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Dear Mr. Figueiredo,

Thank you for your participation in the February-2015 Ring test for PCR laboratories. In the following pages you will find a summary of your results along with those of 20 other participants. As it is customary, the names of the labs have been substituted by a code letter. The letter assigned to your laboratory is the letter "G". On this occasion, we had the participation of laboratories from 11 countries as follows: Brazil (1 laboratory), Colombia (1 laboratory), Ecuador (2 laboratories), India (1 laboratory), Kingdom of Saudi Arabia (4 laboratories), Madagascar (1 laboratory), Malaysia (1 laboratory), Mexico (6 laboratories), Mozambique (2 laboratories), Nicaragua (1 laboratory) and USA (1 laboratory).

The pathogens represented in the panel of samples you received were TSV, YHV, IMNV, NHP (*Hepatobacter penaei*), WSSV and IHNV. Specific pathogen-free (SPF) shrimp tissue that was free of these pathogens was also included in the sample sets for DNA and RNA extraction. Additionally, on this occasion, one of the samples contained more than one pathogen (your sample D-4). A summary of the results from all of the participants is shown in Table 1. If a laboratory did not test for a particular pathogen, the designation "N-An" (Not Analyzed) was used. If no pathogens were detected in the sample, the designation "ND" (Not Detected) was used. If a sample was reported incorrectly, the box appears shaded to indicate the incorrect result. Tables 2 and 3 show summaries of methodology and turnaround times, respectively.

Your laboratory chose to detect the following pathogens: TSV, YHV, IMNV, WSSV, IHNV and NHP (*Hepatobacter penaei*). As you could see in Table 1, your laboratory reported all these DNA and RNA samples correctly. Congratulations.

Thank you for your participation in this 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 Belo Horizonte, Brazil.

Sincerely,

Carlos Pantoja, PhD
Associate Research Professor

Donald V. Lightner, PhD
Professor

Disclosure: This Ring test is intended only as a means to evaluate proficiency in the detection of shrimp pathogens by RT-PCR/PCR. 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.

Table 1. Ring Test of PCR laboratories. FEBRUARY 2015. Summary of results.

Sample Code	Laboratory-A		Laboratory-B		Laboratory-C		Laboratory-D		Laboratory-E	
	Pathogen	Results	Pathogen	Results	Pathogen	Results	Pathogen	Results	Pathogen	Results
R-1	IMNV	IMNV	IMNV	N-An	TSV	N-An	TSV	TSV		PENDING
R-2	TSV	TSV	SPF	N-An	YHV	N-An	IMNV	N-An		PENDING
R-3	SPF	ND	YHV	N-An	SPF	N-An	SPF	ND		PENDING
R-4	YHV	YHV	SPF	N-An	IMNV	N-An	SPF	ND		PENDING
R-5	SPF	ND	TSV	N-An	SPF	N-An	YHV	N-An		PENDING
Correct/Total		5/5		--		--		3/3		PENDING

D-1	NHP-WSSV	WSSV NHP/N-An	SPF	ND	IHHNV	N-An	WSSV	WSSV		PENDING
D-2	WSSV	WSSV	WSSV	WSSV	WSSV	WSSV	NHP-WSSV	WSSV NHP/N-An		PENDING
D-3	SPF	ND	SPF	ND	SPF	ND	IHHNV	IHHNV		PENDING
D-4	IHHNV	IHHNV	IHHNV	N-An	NHP-WSSV	WSSV NHP/N-An	SPF	ND		PENDING
D-5	SPF	ND	NHP-WSSV	WSSN NHP/N-An	SPF	ND	SPF	ND		PENDING
Correct/Total		5/5		4/4		4/4		5/5		PENDING

Sample Code	Laboratory-F		Laboratory-G		Laboratory-H		Laboratory-I		Laboratory-J	
	Pathogen	Results	Pathogen	Results	Pathogen	Results	Pathogen	Results	Pathogen	Results
R-1	SPF	ND	YHV	YHV	SPF	ND	TSV	TSV	IMNV	IMNV
R-2	TSV	TSV	SPF	ND	IMNV	ND	SPF	ND	YHV	YHV
R-3	YHV	YHV	IMNV	IMNV	YHV	ND	SPF	ND	TSV	TSV
R-4	SPF	ND	TSV	TSV	SPF	ND	IMNV	IMNV	SPF	ND
R-5	IMNV	IMNV	SPF	ND	TSV	TSV	YHV	N-An	SPF	ND
Correct/Total		5/5		5/5		3/5		4/4		4/4

D-1	SPF	ND	IHHNV	IHHNV	SPF	ND	WSSV	WSSV	NHP-WSSV	WSSV NHP
D-2	WSSV	WSSV	SPF	ND	IHHNV	N-An	NHP-WSSV	WSSV NHP	IHHNV	IHHNV
D-3	IHHNV	IHHNV	WSSV	WSSV	NHP-WSSV	NHP/N-An WSSV-ND	SPF	ND	SPF	ND
D-4	SPF	ND	NHP-WSSV	NHP WSSV	SPF	ND	IHHNV	IHHNV	WSSV	WSSV
D-5	NHP-WSSV	NHP WSSV	SPF	ND	WSSV	ND	SPF	ND	SPF	ND
Correct/Total		6/6		6/6		2/4		6/6		6/6

(Continues)

Table 1 (Continued). Ring Test of PCR laboratories. FEBRUARY 2015. Summary of results.

Sample Code	Laboratory-K		Laboratory-L		Laboratory-M		Laboratory-N		Laboratory-P	
	Pathogen	Results	Pathogen	Results	Pathogen	Results	Pathogen	Results	Pathogen	Results
R-1	YHV	YHV	SPF	ND	TSV	TSV	SPF	ND	PvNV	N-An
R-2	SPF	ND	YHV	YHV	IMNV	IMNV	TSV	TSV	TSV	ND
R-3	SPF	ND	IMNV	IMNV	SPF	ND	SPF	ND	YHV	YHV IMNV
R-4	TSV	TSV	SPF	ND	YHV	YHV	IMNV	IMNV	SPF	ND
R-5	IMNV	IMNV	TSV	TSV	SPF	ND	YHV	N-An	IMNV	IMNV
Correct/Total		5/5		5/5		5/5		4/4		2/4
D-1	SPF	ND	IHHNV	IHHNV	SPF	ND	SPF	ND	SPF	ND
D-2	WSSV	WSSV	SPF	ND	NHP-WSSV	WSSV NHP	IHHNV	IHHNV	WSSV	WSSV
D-3	IHHNV	IHHNV	WSSV	WSSV	SPF	ND	SPF	ND	IHHNV	IHHNV
D-4	NHP-WSSV	WSSV NHP	SPF	ND	WSSV	WSSV	NHP-WSSV	NHP/N-An WSSV	SPF	ND
D-5	SPF	ND	NHP-WSSV	WSSV NHP	IHHNV	IHHNV	WSSV	WSSV	NHP-WSSV	NHP/N-An WSSV-ND
Correct/Total		6/6		6/6		6/6		5/5		4/5

Sample Code	Laboratory-Q		Laboratory-R		Laboratory-S		Laboratory-T		Laboratory-U	
	Pathogen	Results	Pathogen	Results	Pathogen	Results	Pathogen	Results	Pathogen	Results
R-1	TSV	TSV	IMNV	IMNV	YHV	YHV	TSV	TSV	IMNV	IMNV
R-2	PvNV	PvNV	TSV	TSV	TSV	TSV	YHV	YHV	TSV	TSV
R-3	IMNV	IMNV	PvNV	N-An	IMNV	IMNV	SPF	ND	YHV	YHV
R-4	YHV	YHV	SPF	ND	PvNV	ND	IMNV	IMNV	PvNV	PvNV
R-5	SPF	ND	YHV	YHV	SPF	PvNV	PvNV	PvNV	SPF	ND
Correct/Total		5/5		4/4		3/5		5/5		5/5
D-1	WSSV	WSSV	WSSV	WSSV	IHHNV	IHHNV	WSSV	WSSV	SPF	ND
D-2	SPF	ND	IHHNV	IHHNV	WSSV	WSSV	SPF	ND	IHHNV	NHP IHHNV
D-3	IHHNV	IHHNV	SPF	ND	NHP-WSSV	NHP WSSV	IHHNV	IHHNV	WSSV	WSSV
D-4	NHP-WSSV	NHP WSSV-ND	SPF	ND	SPF	ND	NHP-WSSV	NHP WSSV-ND	NHP-WSSV	WSSV NHP-ND
D-5	SPF	ND	NHP-WSSV	NHP WSSV-ND	SPF	ND	SPF	ND	SPF	ND
Correct/Total		5/6		5/6		6/6		5/6		4/6

(Continues)

Table 1 (Continued). Ring Test of PCR laboratories. FEBRUARY 2015. Summary of results.

Sample Code	Laboratory-V	
	Pathogen	Results
R-1	PvNV	PvNV
R-2	YHV	YHV
R-3	IMNV	IMNV
R-4	SPF	ND
R-5	TSV	TSV
Correct/Total		5/5
D-1	IHHNV	IHHNV
D-2	SPF	ND
D-3	NHP-WSSV	NHP WSSV
D-4	WSSV	WSSV
D-5	SPF	ND
Correct/Total		6/6

Notes:

- 1) N-An=Not analyzed
- 2) ND=Not detected.

Table 2. Summary of PCR/RT-PCR and extraction methods employed by participants in the Ring test for PCR laboratories. FEB -2015.

METHOD	Laboratory-A	Laboratory-B	Laboratory-C	Laboratory-D	Laboratory-E
EXTRACTION					
RNA	Silica Extraction Kit GeneReach Biotech	Not Analyzed	Not Analyzed	IQ2000 Kit	PENDING
DNA	Silica Extraction Kit GeneReach Biotech	IQ2000 Kit	IQ2000 Kit	IQ2000 Kit	PENDING
PRIMERS OR KIT					
TSV	IQREAL TSV Detection Kit	Not Analyzed	Not Analyzed	OIE manual (9992F/9195R)	PENDING
YHV	IQREAL YHV Detection Kit	Not Analyzed	Not Analyzed	Not Analyzed	PENDING
IMNV	IQREAL IMNV Detection Kit	Not Analyzed	Not Analyzed	Not Analyzed	PENDING
PvNV	<i>Not Applicable</i>	<i>Not Applicable</i>	<i>Not Applicable</i>	<i>Not Applicable</i>	<i>Not Applicable</i>
WSSV	IQREAL WSSV Detection Kit	IQ2000 WSSV Kit	IQ2000 WSSV Kit	IQ2000 WSSV Kit	PENDING
NHP	Not Analyzed	Not Analyzed	Not Analyzed	Not Analyzed	PENDING
IHHNV	IQREAL IHHNV Detection Kit	Not Analyzed	Not Analyzed	OIE Manual (389F/389R)	PENDING
ENZYME					
RT-PCR	IQREAL Detection Kits	Not Analyzed	Not Analyzed	High Capacity cDNA Kit Applied Biosystems	PENDING
PCR	IQREAL Detection Kits	IQ2000 WSSV Kit	IQ2000 WSSV Kit	Applied Biosystems	PENDING

(Continues)

Table 2. Summary of PCR/RT-PCR and extraction methods employed by participants in the Ring test for PCR laboratories. FEB-2015.

METHOD	Laboratory-F	Laboratory-G	Laboratory-H	Laboratory-I	Laboratory-J
EXTRACTION RNA	OIE Manual (TRIZOL®)	TRIZOL® LS Reagent Ambion Life Technologies	DSBIO General RNA Extraction Kit	IQ2000 Kits	Qiagen RNeasy minikit
DNA	Macherey-Nagel Kit	Maxwell 16 Tissue DNA Purification Kit (Promega)	Eurofins Gene Spin DNA Isolation Kit	IQ2000 Kits	Wizard Genomic DNA Promega
PRIMERS OR KIT TSV	Navarro et al. (2009) & Tang & Lightner (2004)	OIE Manual (qRT-PCR1004F/1075R) & (RT-PCR9992F/9195R)	OIE Manual (9992F/9195R)	IQ2000 TSV Kit	OIE Manual (9992F/9195R)
YHV	Wijegoonawardane et al (2010)	Aranguren et al. (2012) & OIE Manual (RT-PCR10F/144R)	Unknown method Details not disclosed	Not Analyzed	OIE Manual (10F/144R)
IMNV	Poulos & Lightner (2006)	OIE Manual (qRT-PCR412F/545R)	Real time PCR (?) using primers by Poulos & Lightner 2006	IQ2000 IMNV Kit	Poulos & Lightner (2006)
PvNV	<i>Not Applicable</i>	<i>Not Applicable</i>	<i>Not Applicable</i>	<i>Not Applicable</i>	<i>Not Applicable</i>
WSSV	IQ2000 WSSV Kit & Sritunyalucksana et al. (2006) & Tang & Lightner (2001) & OIE Manual	OIE Manual (qPCR1011F/1079R) (PCR146F/146R)	OIE Manual (using only the second setp primers 146F2/146R2)	IQ2000 WSSV Kit	OIE Manual (146F1/146R1- 146F2/146R2)
NHP	Nunan et al. (2008)	OIE Manual (qPCR1300F/1366R)	Not Analyzed	IQ2000 NHP Kit	Nunan et al. (2008)
IHHNV	OIE Manual & Tang et al. (2007)	OIE Manual (qPCR1608F/1688R)	Not Analyzed	IQ2000 IHHNV Kit	OIE Manual (389F/389R)
ENZYME RT-PCR	- SuperScript One-Step RT- PCR w/Platinum Taq Polymerase (Invitrogen) -Kappa Probe Fast One-Step qRT-PCR Master Mix 2X (KapaBiosystems) -Kapa Sybr Fast LightCycler 480 Master Mix (Kapa Biosystems)	QuantiTec® Virus Kit (Qiagen) OneStep® RT-PCR Kit (Qiagen)	Taq Man by Life Technologies (no other details provided)	IQ2000 Kits	Access RT PCR System Promega
PCR	-Platinum Taq DNA Polymerase (Invitrogen) -Kappa Probe Fast One-Step qPCR Master Mix 2X (KapaBiosystems) -Kapa Sybr Fast LightCycler 480 Master Mix (Kapa Biosystems)	TaqMan Universal PCR Master Mix (Applied Biosystems, Life Technologies) HotStart Taq DNA Polymerase (Qiagen)	Go Taq DNA Polymerase	IQ2000 Kits	Go Taq Hot Start Promega

(Continues)

Table 2. Summary of PCR/RT-PCR and extraction methods employed by participants in the Ring test for PCR laboratories. FEB-2015.

METHOD	Laboratory-K	Laboratory-L	Laboratory-M	Laboratory-N	Laboratory-P
EXTRACTION RNA	IQ2000 Kits Genereach Biotechnologies	TACO Nucleic acid extraction system	IQ2000 Kits Genereach Biotechnologies	PureLink RNA Minikit (Life Technologies; Ambion)	Magnalyser Green Beads (Roche) MagNAPure Cpmpact RNA Isolation Kit (Roche)
DNA	IQ2000 Kits Genereach Biotechnologies	TACO Nucleic acid extraction system	IQ2000 Kits Genereach Biotechnologies	OIE Manual (Lysis solution/CTAB)	High Pure Tissue Kit (Roche)
PRIMERS OR KIT TSV	IQ2000 TSV Kit IQ Real TSV Kit Nunan et al. 1998	IQ Real TSV Kit	IQ2000 TSV Kit	OIE Manual (9992F/9195R)	OIE Manual (9992F/9195R)
YHV	IQ2000 YHV Kit IQ Real YHV Kit Wijegoonawardane et al. 2008	IQ Real YHV Kit	IQ2000 YHV Kit	Not Analyzed	OIE Manual (Protocol 3)
IMNV	IQ200 IMNV Kit IQ Real IMNV Kit Poulos & Lightner (2006)	IQ Real IMNV Kit	IQ200 IMNV Kit	Poulos & Lightner (2006)	Poulos & Lightner (2006)
PvNV	<i>Not Applicable</i>	<i>Not Applicable</i>	<i>Not Applicable</i>	<i>Not Applicable</i>	Not Analyzed
WSSV	IQ2000 WSSV Kit IQ Real WSSV Kit Nunan & Lightner 2011	IQ Real WSSV Kit	IQ2000 WSSV Kit	IQ2000 WSSV Kit	OIE Manual (146F1/146R1- 146F2/146R2)
NHP	IQ2000 NHP Kit IQ Real NHP Kit Nunan et al. 2008	IQ Real NHP Kit	IQ2000 NHP Kit	Not Analyzed	Not Analyzed
IHHNV	IQ2000 IHHNV Kit IQ Real IHHNV Kit Tang et al. 2007	IQ Real IHHNV Kit	IQ2000 IHHNV Kit	OIE Manual (77012F/77353R) & (389F/389R)	OIE Manual (389F/389R) & (77012F/77353R) & (392F/392R)
ENZYME RT-PCR	Life Technologies/Kappa Biosystem	IQ Real Kits	IQ2000 Kits	SuperScript III First Strand Synthesis Super Mix (Life Technologies; Invitrogen)	MMLV or AMV Reverse Transcriptase Hot-Start DNA Polymerase (QIAGEN)
PCR	Life Technologies/Kappa Biosystem	IQ Real Kits	IQ2000 Kits	IQ2000 Kit AmpliTaq Gold 360 Master Mix (Life Technologies; Invitrogen)	FastStart Taq DNA Polymerase dNTPack (Roche)

(Continues)

Table 2. Summary of PCR/RT-PCR and extraction methods employed by participants in the Ring test for PCR laboratories.

FEB-2015.

METHOD	Laboratory-Q	Laboratory-R	Laboratory-S	Laboratory-T	Laboratory-U
EXTRACTION RNA	QIAamp Viral RNA Mini Kit (QIAGEN)	OIE Manual (TRIZOL®)	Total RNA Isolation System (Promega)	Maxwell 16 Tissue LEV Total RNA Purification (Promega)	IQ2000 Kits
DNA	DNeasy Blood and Tissue Kit (QIAGEN)	High Pure PCR Kit (Roche)	Wizard Genomic DNA Purification Kit (Promega)	Maxwell 16 LEV DNA Purification (Promega)	IQ2000 Kits
PRIMERS OR KIT TSV	OIE Manual (9992F/9195R)	OIE Manual (qRT-PCR1004F/1075R) & (RT-PCR9992F/9195R)	OIE Manual (9992F/9195R)	OIE Manual (9992F/9195R)	IQ2000 TSV Kit
YHV	OIE Manual (Protocol 3)	OIE Manual (Protocol 3)	OIE Manual (Protocol?)	OIE Manual (RT-PCR10F/144R)	IQ2000 YHV Kit
IMNV	Poulos & Lightner (2006)	Poulos & Lightner (2006)	OIE Manual [Poulos & Lightner (2006)]	OIE Manual Real time RT-PCR modified from Andrade et al. (2007)	IQ2000 IMNV Kit
PvNV	Tang et al. (2007)	Not Analyzed	Tang et al. (2007)	Tang et al. (2007)	IQ2000 PvNV Kit
WSSV	OIE Manual (146F1/146R1-146F2/146R2)	OIE Manual (146F1/146R1-146F2/146R2)	OIE Manual (146F1/146R1-146F2/146R2)	Nunan & Lightner (2011)	IQ2000 WSSV Kit
NHP	Not disclosed	OIE Manual (qPCR1300F/1366R)	OIE Manual (Nunan et al. 2008)	OIE Manual (Nunan et al. 2008)	IQ2000 NHP Kit
IHHNV	OIE Manual (392F/392R)	OIE Manual (392F/392R)	OIE Manual (392F/392R)	OIE Manual (389F/389R)	IQ2000 IHHNV Kit
ENZYME RT-PCR	One Step RT-PCR Kit (QIAGEN) Real time ready RNA Virus Master (Roche)	One Step RT-PCR kit (QIAGEN) Real time ready RNA Virus Master (Roche)	ImProm-II™ Reverse Transcription System (Promega)	SuperScript III One-Step RT-PCR System w/Platinum Taq DNA Polymerase TaqMan Fast Virus 1-step Master Mix	IQ2000 Kits
PCR	Not Disclosed	Taq PCR Core (QIAGEN) Real Time Ready RNA Virus Master (Roche)	GoTaq® Flexi DNA Polymerase	Ready-to-go beads (GE Healthcare)	IQ2000 Kits

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Table 2. Summary of PCR/RT-PCR and extraction methods employed by participants in the Ring test for PCR laboratories.

FEB-2015.

METHOD	Laboratory-V
EXTRACTION	
RNA	TACO Nucleic acid extraction system
DNA	TACO Nucleic acid extraction system
PRIMERS OR KIT	
TSV	IQ2000 TSV Kit
YHV	IQ2000 YHV Kit
IMNV	IQ2000 IMNV Kit
PvNV	IQ2000 PvNV Kit
WSSV	IQ2000 WSSV Kit
NHP	IQ2000 NHP Kit
IHHNV	IQ2000 IHHNV Kit
ENZYME	
RT-PCR	IQ2000 Kits
PCR	IQ2000 Kits

Table 3. Summary of turn around times. Ring test for PCR laboratories. FEBRUARY 2015.

SAMPLE SET CODE LETTER	SAMPLE SHIPMENT DATE (mm/dd/yy)	SAMPLE RECEIPT DATE (mm/dd/yy)	SUBMISSION OF RESULTS (mm/dd/yy)	TOTAL WORKING DAYS
A	02/23/15	03/01/15	03/06/15	5
B	02/16/15	02/23/15	03/16/15	15
C	02/16/15	02/24/15	03/06/15	8
D	02/17/15	02/23/15	02/27/15	4
E	02/16/15	02/25/15	PENDING	PENDING
F	02/17/15	02/23/15	03/04/15	7
G	02/26/15	03/06/15	03/12/15	4
H	02/24/15	03/03/15	03/26/15	17
I	02/17/15	02/26/15	03/04/15	4
J	02/17/15	02/23/15	02/28/15	5
K	02/16/15	02/23/15	02/28/15	5
L	02/17/15	03/01/15	03/06/15	5
M	02/16/15	02/27/15	03/09/15	6
N	02/18/15	02/26/15	03/05/15	5
P	02/17/15	02/26/15	03/06/15	6
Q	02/17/15	02/26/15	03/06/15	6
R	02/17/15	02/26/15	03/04/15	4
S	02/13/15	02/17/15	03/04/15	11
T	02/16/15	02/16/15	02/23/15	5
U	02/13/15	02/26/15	03/10/15	8
V	02/17/15	03/02/15	03/06/15	4