

AQUACULTURE PATHOLOGY
OFFICE PHONE: (520)621-4438
FAX: (520)621-4899

March 19, 2013

Prof. Henrique Cesar Pereira Figueredo
AQUACEN-Animal Health,
Ministry of Fisheries and Aquaculture
Veterinary School – Federal University of Minas Gerais-UFMG
Av. Antonio Carlos No. 6627, Campus UFMG Pampulha,
Belo Horizonte-MG, postal code 31.123-970
BRAZIL

Email: 

Dear Professor Pereira,

Thank you for your participation in the February 2013 Ring test for PCR laboratories. In the following pages you will find a summary of your results, along with those of 17 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 "K". On this occasion, we had the participation of laboratories from 10 countries as follows: Belize (1 laboratory), Brazil (1 laboratory), Ecuador (2 laboratories), India (1 laboratory), Madagascar (1 laboratory), Mexico (6 laboratories), Nicaragua (1 laboratory), Saudi Arabia (3 laboratories), Thailand (1 laboratory) and USA (1 laboratory).

The pathogens represented in the panel of samples you received were TSV, YHV, IMNV, NHP, 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. 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 turn around times, respectively.

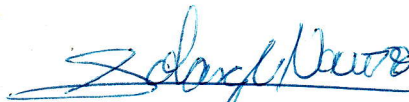
Your laboratory chose to detect the following pathogens: TSV, YHV, IMNV, WSSV and IHNV. As you could see in Table 1, your laboratory reported all of these DNA and RNA samples correctly. Additionally, your turn around time was among the shortest. Congratulations.

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



Solangel Navarro
Research Specialist



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 2013. Summary of results.

Sample Code	Laboratory-A		Laboratory-B		Laboratory-C		Laboratory-D		Laboratory-E		Laboratory-F		Laboratory-G	
	Pathogen	Results	Pathogen	Results	Pathogen	Results	Pathogen	Results	Pathogen	Results	Pathogen	Results	Pathogen	Results
R-1	IMNV	N-An	TSV	TSV	YHV	TSV	YHV	N-An	IMNV	IMNV	SPF	ND	TSV	TSV
R-2	TSV	ND	IMNV	IMNV	SPF	TSV	TSV	TSV	SPF	ND	YHV	YHV	IMNV	IMNV
R-3	YHV	N-An	YHV	YHV	TSV	TSV	IMNV	IMNV	SPF	ND	IMNV	IMNV	SPF	ND
R-4	SPF	ND	SPF	ND	IMNV	TSV&IMNV	SPF	ND	TSV	TSV	SPF	ND	YHV	N-An
R-5	SPF	ND	SPF	ND	SPF	ND	SPF	ND	YHV	YHV	TSV	TSV	SPF	ND
Correct/Total		2/3		5/5		2/5		4/4		5/5		5/5		4/4
D-1	SPF	ND	WSSV	WSSV	WSSV	N-An	SPF	ND	IHHNV	IHHNV	IHHNV	IHHNV	SPF	ND
D-2	WSSV	WSSV	SPF	ND	IHHNV	N-An	IHHNV	IHHNV	WSSV	WSSV	SPF	ND	WSSV	WSSV
D-3	IHHNV	N-An	IHHNV	IHHNV	SPF	N-An	WSSV	WSSV	SPF	ND	WSSV	WSSV	IHHNV	IHHNV
D-4	NHP	N-An	SPF	ND	SPF	N-An	NHP	NHP	SPF	N-An	SPF	ND	SPF	ND
D-5	SPF	N-An	NHP	NHP	NHP	N-An	SPF	ND	NHP	N-An	NHP	NHP	NHP	NHP
Correct/Total		2/2		5/5				5/5		3/3		5/5		5/5

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

(Continues)

Table 1 (Continued). Ring Test of PCR laboratories. February 2013. Summary of results.

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

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. February 2013.

METHOD	Laboratory-A	Laboratory-B	Laboratory-C	Laboratory-D	Laboratory-E
EXTRACTION RNA	IQ2000 RNA	OIE 2006 Diagnostic Manual	Roche High Pure RNA Tissue kit	TACO™ Nucleic Acid Automatic Extraction System by GeneReach Biotechnology Corp.	IQ2000 RNA
DNA	IQ2000 DNA	Nucleospin Tissue kit (Macherey-Nagel)	<i>NOT ANALYZED</i>	TACO™ Nucleic Acid Automatic Extraction System by GeneReach Biotechnology Corp.	IQ2000 DNA
PRIMERS OR KIT TSV	IQ2000 TSV	Navarro et al.(2009), IQ2000 TSV kit & TaqMan Real Time by Tang & Lightner 2004	Not disclosed	IQ2000 TSV	IQ2000 TSV
YHV	<i>NOT ANALYZED</i>	IQ2000 YHV & SYBR Green Real Time by Wijegoonawardane et al. 2010	<i>NOT ANALYZED</i>	<i>NOT ANALYZED</i>	IQ2000 YHV/GAV
IMNV	<i>NOT ANALYZED</i>	Poulos et al. 2006	Not disclosed	IQ2000 IMNV	IQ2000 IMNV
PvNV	<i>NOT APPLICABLE</i>	<i>NOT APPLICABLE</i>	<i>NOT APPLICABLE</i>	<i>NOT APPLICABLE</i>	<i>NOT APPLICABLE</i>
WSSV	IQ2000 WSSV	IQ2000 WSSV, OIE 20011 Diagnostic Manual & TaqMan Real Time by Sritunyalucksana et al 2006	<i>NOT ANALYZED</i>	IQ2000 WSSV	IQ2000 WSSV
NHP	<i>NOT ANALYZED</i>	Nunan et al. 2008	<i>NOT ANALYZED</i>	IQ2000 NHP	<i>NOT ANALYZED</i>
IHHNV	<i>NOT ANALYZED</i>	OIE 2006 Diagnostic Manual & SYBR Green Real Time by Tang et al. 2007	<i>NOT ANALYZED</i>	IQ2000 IHHNV	OIE Diagnostic Manual (77012F/77353R)
ENZYME RT-PCR	TSV: IQ2000 kit	TSV: One step RT-PCR w/Platinum Taq (Invitrogen) KAPA Probe FAST One-Step qRT-PCR (KAPA Biosystems) YHV: KAPA SYBR FAST (KAPA Biosystems) IMNV: SuperScriptII One-Step RT-PCR (Invitrogen) & Platinum Taq (Invitrogen)	GeneAmp EZ rTth RNA PCR kit	IQ2000 kits	IQ2000 kits
PCR	WSSV: IQ2000 kit	KAPA Probe FAST qPCR & KAPA SYBER FAST (KAPA Biosystems) Platinum Taq (Invitrogen)	<i>NOT ANALYZED</i>	IQ2000 kits	WSSV: IQ2000 kit IHHNV: Taq DNA Polymerase (Invitrogen)

(Continues)

Table 2 (Continued). Summary of PCR/RT-PCR and extraction methods employed by participants in the Ring test for PCR laboratories. February 2013.

METHOD	Laboratory-F	Laboratory-G	Laboratory-H	Laboratory-I	Laboratory-J
EXTRACTION RNA	IQ2000 RNA	IQ2000 RNA	TACO™ Nucleic Acid Automatic Extraction System by GeneReach Biotechnology Corp.	IQ2000 RNA	Qiagen RNeasy Mini kit
DNA	IQ2000 DNA	IQ2000 DNA	TACO™ Nucleic Acid Automatic Extraction System by GeneReach Biotechnology Corp.	IQ2000 DNA	Qiagen DNeasy Blood & Tissue kit
PRIMERS OR KIT TSV	IQ2000 TSV	IQ2000 TSV	IQ2000 Real time TSV	IQ2000 TSV	OIE Manual 2012
YHV	IQ2000 YHV	<i>NOT ANALYZED</i>	IQ2000 YHV	IQ2000 YHV	<i>NOT ANALYZED</i>
IMNV	IQ2000 IMNV	IQ2000 IMNV	IQ2000 IMNV	IQ2000 IMNV	<i>NOT ANALYZED</i>
PvNV	<i>NOT APPLICABLE</i>	<i>NOT APPLICABLE</i>	<i>NOT APPLICABLE</i>	<i>NOT APPLICABLE</i>	<i>NOT APPLICABLE</i>
WSSV	IQ2000 WSSV	IQ2000 WSSV	IQ2000 Real time WSSV	IQ2000 WSSV	OIE Manual 2012 (Second step)
NHP	IQ2000 NHP	IQ2000 NHP	IQ2000 NHP	IQ2000 NHP	<i>NOT ANALYZED</i>
IHHNV	IQ2000 IHHNV	IQ2000 IHHNV	IQ2000 IHHNV	IQ2000 IHHNV	<i>NOT ANALYZED</i>
ENZYME RT-PCR	IQ2000 kits	IQ2000 kits	TSV: IQ2000 Real time YHV, IMNV: IQ2000 kits	IQ2000 kits	EZ rTth RNA PCR kit (Applied Biosystems)
PCR	IQ2000 kits	IQ2000 kits	WSSV: IQ2000 Real time IHHNV, NHP: IQ2000 kits	IQ2000 kits	GoTaq DNA Polymerase (Promega)

(Continues)

Table 2 (Continued). Summary of PCR/RT-PCR and extraction methods employed by participants in the Ring test for PCR laboratories. February 2013.

METHOD	Laboratory-K	Laboratory-L	Laboratory-M	Laboratory-N	Laboratory-P
EXTRACTION					
RNA	Trizol LS Reagent (Invitrogen)	Not disclosed	IQ2000 RNA	IQ2000 RNA	IQ2000 RNA
DNA	Qiagen DNeasy Blood & Tissue kit	IQ2000 DNA	IQ2000 DNA	IQ2000 DNA	IQ2000 DNA
PRIMERS OR KIT					
TSV	OIE Manual 2012	Not disclosed	IQ2000 TSV	IQ2000 TSV	IQ2000 TSV
YHV	OIE manual 2012	Not disclosed	IQ2000 YHV	IQ2000 YHV	IQ2000 YHV
IMNV	OIE Manual (2012) Real time RT-PCR	Not disclosed	IQ2000 IMNV	IQ2000 IMNV	IQ2000 IMNV
PvNV	<i>NOT APPLICABLE</i>	Not disclosed	IQ2000 PvNV	Tang et al. (2007)?	<i>NOT ANALYZED</i>
WSSV	OIE Manual (2012) Real time PCR	Not disclosed	IQ2000 WSSV	Nunan & Lightner (2011)	IQ2000 WSSV & Kimura method with Kimura's primers
NHP	<i>NOT ANALYZED</i>	Not disclosed	IQ2000 NHP	Nunan et al. (2008)	IQ2000 NHP
IHHNV	OIE Manual (2012) Real time PCR	Not disclosed	IQ2000 IHHNV	A UofA method that produces a fragment ~300bp	IQ2000 IHHNV
ENZYME					
RT-PCR	TSV, YHV: HotStar Taq DNA Polymerase (Qiagen) IMNV: QuantiTect Virus (Qiagen)	Not disclosed	IQ2000 Kits	TSV, YHV, IMNV: IQ2000 kits PvNV: QuantiTect Reverse transcription kit (Qiagen)	IQ2000 Kits
PCR	WSSV, IHHNV: QuantiTect Virus (Qiagen)	Not disclosed	IQ2000 Kits	Not disclosed	WSSV: IQ2000 kit & Taq Pol (Invitrogen) NHP: IQ2000 kit

(Continues)

Table 2 (Continued). Summary of PCR/RT-PCR and extraction methods employed by participants in the Ring test for PCR laboratories. February 2013.

METHOD	Laboratory-Q	Laboratory-R	Laboratory-S
EXTRACTION RNA		Viral RNA Isolation Nucleospin RNA Virus kit (Macherey-Nagel)	Silica_Extraction kit (GeneReach)
DNA		Genomic DNA from Tissue Nucleospin Tissue kit (Macherey-Nagel)	Silica_Extraction kit (GeneReach)
PRIMERS OR KIT TSV		OIE Manual (2012)	IQ REAL TSV Quantitative System
YHV		OIE Manual (2012)	IQ REAL YHV Quantitative System
IMNV		OIE Manual (2012)	IQ REAL IMNV Quantitative System
PvNV		<i>NOT ANALYZED</i>	<i>NOT APPLICABLE</i>
WSSV		OIE Manual (2012)	Nunan & Lightner (2011)
NHP-B		<i>NOT ANALYZED</i>	Nunan et al. (2008)
IHHNV		OIE Manual (2012)	Tang et al. (2006)
ENZYME RT-PCR		Tetro OneStep kit (Bioline)	IQ REAL Kits
PCR		Taq PCR Core kit (Qiagen)	Not disclosed

Table 3. Summary of turn around times. Ring test for PCR laboratories. February 2013.

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	02/18/13	02/24/13	02/27/13	3
B	02/13/13	02/22/13	03/05/13	7
C	02/13/13	02/14/13	03/06/13	14
D	02/18/13	03/04/13	03/11/13	5
E	02/13/13	02/18/13	02/23/13	5
F	02/13/13	02/25/13	02/28/13	3
G	02/13/13	02/26/13	03/16/13	14
H	02/13/13	02/24/13	03/02/13	6
I	02/13/13	02/19/13	02/25/13	4
J	02/13/13	02/17/13	02/18/13	1
K	02/14/13	02/19/13	02/25/13	4
L	02/22/13	02/25/13	03/02/13	5
M	02/22/13	02/25/13	03/05/13	7
N	02/22/13	02/25/13	03/02/13	5
P	02/12/13	02/18/13	02/24/13	6
Q	02/22/13	03/01/13	PENDING	
R	02/13/13	03/01/13	03/06/13	3
S	02/13/13	02/18/13	02/22/13	4