



March 21, 2016

Mrs. Omara Escobar  
Service Aqua, LLC  
10880 NW 138<sup>th</sup> Street Bay #5  
Hiialeah Gardens, FL 33018  
USA

**Case: 16-155**

E-mail: [sales@serviceaqua.com](mailto:sales@serviceaqua.com)

Dear Mrs. Escobar:

The PCR and RT-PCR tests you requested for the detection of WSSV, IHNV, TSV, YHV, IMNV, PvNV, NHP-B, *V. parahaemolyticus* and AHPND/EMS have been completed. The sample of Topsy Frozen Marine – SPF Polychaetes *N. virens* (Lot# SAQ0316) arrived on March 15<sup>th</sup>, 2016 in good condition. Information about sampling location and date of collection was not provided. Representative samples (45mg to 48mg) were collected for DNA and RNA extractions. WSSV, IHNV, TSV, YHV, IMNV, PvNV, NHP-B, *V. parahaemolyticus* and AHPND/EMS were not detected in the sample tested. The testing was completed on March 21<sup>st</sup>, 2016. A summary of the tests and results is provided on the following page.

We hope that this information will be helpful to you. If there are any questions regarding this case please feel free to contact us.

**UAZ Policy on certification:** This report provides our findings on the samples submitted to our laboratory for examination, health status evaluation, disease diagnosis, or pathogen detection. It is our policy and intent to perform the most appropriate assay(s) for the determination of the health/pathogen status of all specimens submitted to our laboratory. However, this report in no way constitutes a stock or facility "certification" or a "certificate" of health/pathogen status for the sample(s) tested or for the stocks, or facility, from which the sample(s) were derived.

**PCR: disclaimer:** This report provides our findings on the samples submitted to our laboratory for pathogen detection. The PCR assay used by this laboratory for the detection of shrimp pathogens is a research tool. The results should be considered as experimental and tentative. Whenever possible, PCR results should be confirmed by alternative assay. This report in no way constitutes a stock or facility "certification" or a "certificate" of health/pathogen status for the sample(s) tested or for the stocks, or facility, from which the sample(s) were derived.

The **UAZ Aquaculture Pathology Lab** is a **OIE** (Office International des Epizooties or the Organization of World Animal Health Organization) Reference Laboratory for White Spot Disease, Taura Syndrome, Infectious Hypodermal and Hematopoietic Necrosis, Spherical Baculovirus, Tetrahedral Baculovirus, Necrotizing Hepatopancreatitis Bacterium, and Infectious Myonecrosis, and a **U.S.D.A. A.P.H.I.S.** Approved Laboratory for export testing for White Spot Disease, Taura Syndrome, Infectious Hypodermal and Hematopoietic Necrosis, Spherical Baculovirus, Tetrahedral Baculovirus, Infectious Myonecrosis, and Yellowhead Disease.

Sincerely yours,

Irina Maskaykina  
Research Specialist

Kathy Tang-Nelson, Ph.D.  
Associate Research Professor

**Case: 16-155**

**Tests Requested:**

TSV, YHV, IMNV, P<sub>v</sub>NV  
 WSSV, IHNV  
 AHPND/EMS, NHP-B, *V. parahaemolyticus*

**Sample Type:**

Frozen polychaetes  
 Frozen polychaetes  
 Frozen polychaetes

**Species:**

*N. virens*  
*N. virens*  
*N. virens*

**Protocols:**

YHV: RT-PCR described by Wongteerasupaya, et al. (DAO, 1997, 31:181-186)  
 TSV: RT-PCR described by Nunan, et al. (DAO, 1998, 34:87-91)  
 WSSV: PCR protocol described by Nunan & Lightner (J Vir Methods, 2011, 171:318-321)  
 IHNV: PCR described by Tang et al. (Virus Research, 2006, 118: 185-191)  
 NHP-B: PCR method described by Aranguren et al. (Aquaculture 2010, 307:187-192)  
 Real time IMNV RT-PCR: modified from Andrade, et al. (Aquaculture. 2007, 264: 9-15)  
 Real time P<sub>v</sub>NV: protocol developed in this laboratory, unpublished.  
 AHPND/EMS: PCR protocol described by Han, et al. (DAO 2015, 113:33-40)  
*V. parahaemolyticus*: PCR protocol described by Kim, et al. (J Clin Microbiology, 1999, 37:1173-1177)

**Table:** PCR and RT-PCR results

UAZ #	Sample Ref	WSSV	IHNV	YHV	TSV	IMNV	P <sub>v</sub> NV	AHPND/EMS	<i>V. parahaemolyticus</i>	NHP-B
16-155	Topsy Frozen Marine SPF Polychaetes <i>N. virens</i> Lot# SAQ0316	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected

**Conclusion:** WSSV, IHNV, TSV, YHV, IMNV, P<sub>v</sub>NV, NHP-B, *V. parahaemolyticus* and AHPND/EMS were not detected in the sample tested.