

AQUACULTURE PATHOLOGY
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December 9, 2008

Dr. Allen Riggs
State of Hawaii
Anuenue Fisheries Research Center
Division of Aquatic Resources
Dept. of Land & Natural Resources
1039 Sand Island Parkway
Honolulu, HI 96819-4347

Case: 08-303

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Dear Dr. Riggs:

The tests you requested for the detection of WSSV, IHHNV, YHV, IMNV, MBV, BP & TSV by PCR have been completed. The 18 vials of *Litopenaeus vannamei* pleopods and hepatopancreas (your ADP # 08-222) arrived on December 8th, 2008 in good condition. Five pleopods or hepatopancreas were taken from each of the vials (19 mg to 40 mg). The samples were collected for DNA and RNA extractions. WSSV, YHV, TSV, IMNV, MBV, BP & IHHNV were Not Detected in any of the samples tested. A summary of the tests and results is provided on the following page.

We hope that this information will be helpful to you. A hard copy of this report will be mailed to you. The fee for these tests is \$972.00 (18 sets of tests WSSV/IHHNV, TSV/YHV and MBV/BP @ \$47.00 each, and 6 sets of tests for IMNV @ \$21.00 each). A separate invoice will be sent 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.

Sincerely yours,

Ms. Solangel Navarro
Research Specialist

D.V. Lightner Ph.D.
Professor

| <u>Test(s) Requested:</u> | <u>Sample Type:</u> | <u>Species:</u> |
|---------------------------|--|--------------------|
| YHV | 6 vials of pleopods in 95% ethanol | <i>L. vannamei</i> |
| TSV | 6 vials of pleopods in 95% ethanol | <i>L. vannamei</i> |
| IMNV | 6 vials of pleopods in 95% ethanol | <i>L. vannamei</i> |
| WSSV | 6 vials of pleopods in 95% ethanol | <i>L. vannamei</i> |
| IHHNV | 6 vials of pleopods in 95% ethanol | <i>L. vannamei</i> |
| MBV | 6 vials of hepatopancreas in 95% ethanol | <i>L. vannamei</i> |
| BP | 6 vials of hepatopancreas in 95% ethanol | <i>L. vannamei</i> |

Protocols:

YHV: RT-PCR described by Tang and Lightner (DAO, 1999, 35:165-173)
 TSV: RT-PCR described by Nunan, et al. (DAO, 1998, 34:87-91)
 WSSV: Two-step nested PCR modified method from Lo, et al (DAO, 1996, 25:133-283)
 BP: PCR protocol developed in this laboratory base on Gen Bank sequence #DQ49617
 MBV: PCR protocol described by Surachetpong et al (Aquaculture, 2005, 249:69-75)
 IMNV: RT-PCR nested protocol described by Poulos and Lightner (DAO,2006,73:69-72)
 IHHNV: PCR described by Nunan, et al. (Marine Biotechnology, 2000, 2: 319-328)

Table:

| UAZ # | Sample Ref | WSSV | IHHNV | TSV | YHV | IMNV | MBV | BP |
|-----------|-----------------------------|-------|-------|-------|-------|-------|--------------|--------------|
| 08-303/A1 | 08-222/A1 Hepatopancreas | ----- | ----- | ----- | ----- | ----- | Not Detected | Not Detected |
| 08-303/A2 | 08-222/A2 Hepatopancreas | ----- | ----- | ----- | ----- | ----- | Not Detected | Not Detected |
| 08-303/A3 | 08-222/A3 Hepatopancreas | ----- | ----- | ----- | ----- | ----- | Not Detected | Not Detected |
| 08-303/A4 | 08-222/A4 Hepatopancreas | ----- | ----- | ----- | ----- | ----- | Not Detected | Not Detected |
| 08-303/A5 | 08-222/A5 Hepatopancreas | ----- | ----- | ----- | ----- | ----- | Not Detected | Not Detected |
| 08-303/A6 | 08-222/A6 Hepatopancreas | ----- | ----- | ----- | ----- | ----- | Not Detected | Not Detected |

| UAZ # | Sample Ref | WSSV | IHHNV | TSV | YHV | IMNV | MBV | NHP |
|-----------|-------------------------|--------------|--------------|--------------|--------------|--------------|-------|-------|
| 08-303/B1 | 08-222/B1 5 pleopods | ----- | ----- | Not Detected | Not Detected | Not Detected | ----- | ----- |
| 08-303/B2 | 08-222/B2 5 pleopods | ----- | ----- | Not Detected | Not Detected | Not Detected | ----- | ----- |
| 08-303/B3 | 08-222/B3 5 pleopods | ----- | ----- | Not Detected | Not Detected | Not Detected | ----- | ----- |
| 08-303/B4 | 08-222/B4 5 Pleopods | ----- | ----- | Not Detected | Not Detected | Not Detected | ----- | ----- |
| 08-303/B5 | 08-222/B5 5 Pleopods | ----- | ----- | Not Detected | Not Detected | Not Detected | ----- | ----- |
| 08-303/B6 | 08-222/B6 5 pleopods | ----- | ----- | Not Detected | Not Detected | Not Detected | ----- | ----- |
| 08-303/C1 | 08-222/C1 5 pleopods | Not Detected | Not Detected | ----- | ----- | ----- | ----- | ----- |
| 08-303/C2 | 08-222/C2 5 pleopods | Not Detected | Not Detected | ----- | ----- | ----- | ----- | ----- |
| 08-303/C3 | 08-222/C3 5 Pleopods | Not Detected | Not Detected | ----- | ----- | ----- | ----- | ----- |
| 08-303/C4 | 08-222/C4 5 Pleopods | Not Detected | Not Detected | ----- | ----- | ----- | ----- | ----- |
| 08-303/C5 | 08-222/C5 5 Pleopods | Not Detected | Not Detected | ----- | ----- | ----- | ----- | ----- |
| 08-303/C6 | 08-222/C6 5 pleopods | Not Detected | Not Detected | ----- | ----- | ----- | ----- | ----- |

Conclusion: WSSV, YHV, TSV, IMNV, MBV, BP & IHHNV were Not Detected in any of the samples tested.