

School of Animal & Comparative Biomedical Sciences Bldg. #90, Room 102 1117 E. Lowell Street, Tucson, Arizona 85721-0090 Phone: 520-621-4438; Email: <u>aquapath@cals.arizona.edu</u>



Date: 9-18-2024

Dear Colleague,

This letter is to notify you of our next inter-laboratory calibration (Ring Test) for detection of shrimp pathogens by PCR/RT-PCR. The Ring test is scheduled to begin **February 15, 2025**. As in previous exercises, there will be no prescribed tests and each participating laboratory will employ the same PCR/RT-PCR procedures they routinely use to analyze clinical samples.

Each laboratory will be provided with a panel of 10 coded tissue samples. The following pathogens will be present in the panel: WSSV, IHHNV, NHP, AHPND, EHP, TSV, YHV, IMNV and PvNV; there will also be SPF tissue. **Please be aware that starting with this test we are no longer able to supply samples for DIV-1.** PvNV will now be included by default in all tests, and there will no longer be an additional charge for PvNV. More than one pathogen may be present in a given sample. Your laboratory will test only for the pathogens that you are set up to analyze. For example, if you are only set up to test for WSSV and TSV, then you would analyze these samples only for WSSV and TSV. If you are interested, you are welcome to test all pathogens. You will only need to specify in your report which pathogens were tested. A standard format for the report will be sent to you by e-mail immediately after you confirm receipt of the samples.

While there are many reasons why laboratories are not able to successfully complete the Ring Test, the most common is the mishandling of samples. All of these provided samples (except for those with SPF tissue) include nucleic acid positive for pathogens, and without proper separation and care they can easily cross-contaminate each other due to the sensitivity of PCR/qPCR testing. This test will not just help a laboratory determine if their PCR/qPCR cycling conditions and primers are working; it will also help determine if their sample reception procedures, cleaning procedures, and laboratory layout are sufficient to prevent cross-contamination. Please keep this in mind as you consider your participation in this test!

The participation fee for this Ring Test is \$1,100.00 USD, which includes one panel of 10 samples, analysis and interpretation of your results, and preparation of a final report, with recommendations when necessary. Shipping costs are not included. If you wish to provide us with a FEDEX or DHL account number, we will ship the samples to you at no additional charge. Otherwise shipping charges will be added to the cost. Full payment for participation in the Ring Test (including any shipping costs) will be required before we can send you the samples. The last day to submit payment is **February 1, 2025**.

Shipping of the samples to the laboratories has been scheduled for February 14<sup>th</sup>, 2024. The window for results submission will be from: February 16<sup>th</sup> to March 31<sup>st</sup>, 2025. A final report will be provided to you by: April 30<sup>th</sup>.

In the following pages, we have included an application form for you to provide the information necessary for invoicing, shipping the samples, and e-mailing/mailing the final report. Due to increasing costs, we will no longer provide physical copies of results unless requested in the application. If you are interested in participating, please return the application form to us by e-mail as soon as possible. We limit the number of participating laboratories to 30, and we reach this limit relatively fast. Once your application is received, an invoice will be prepared and sent to you by e-mail.

Please note – we have several updated policies surrounding falsification or collusion of results and the use of third parties for procuring the Ring Test. All laboratories are required to submit images of either gel pictures or amplification plots along with descriptions of their methods. If these are found to be incongruous, or if there is suspected falsification (i.e., doctored images), the laboratory will investigate. If clear evidence is found of falsification or collusion, that client (and any other participants found to be involved in collusion) will not receive their results for that test and will be banned permanently from participating in future tests.

Similarly, the APL does not allow the use of third parties to procure the Ring Test. All inquiries and purchases must be made directly through us. If you must use a third party for customs or shipping purposes, we will require additional documentation from you. These rules are to preserve the integrity of the test and ensure that all labs are participating fairly.

We also maintain a results appeal process. If you are unsatisfied with your results, please submit in writing (preferably by email, though physical mail will also be accepted) your desire to appeal. You will be provided with a form which will outline the evidence you are required to supply. If found to be sufficient, your report will be revised to reflect the change in results.

Some countries have very strict import regulations. We strongly recommend that, <u>before</u> committing to participating in this Ring test, you find out first if there are any special requirements for importation of these samples. If necessary, please obtain any special permits and send us a copy, so that we may include it with your samples. Help us avoid delays, or even rejection, by customs and other inspection authorities upon arrival of the samples to your country. The Ring test samples have been previously preserved in 95% ethanol, they can be considered innocuous and do not represent a hazard to humans, plants or animals.

Best regards,

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Arun K. Dhar, Ph.D. Professor Aquaculture Pathology Laboratory Director

**Confidentiality Statement:** Please note – while your data will be anonymized and not identifiable to you, it will be shared with other participants. All labs will be able to view the performance and methods of all other labs within the final report tables. Your lab's contact information and identity will remain confidential and will not be shared with any other participants.