INTRODUCTION

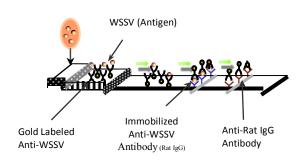
Outbreaks of white spot syndrome (WSS) have been known to cause serious mortality rate to farmed shrimp. It has been occurring since 1992 in the East Asia. WSS has spread widely, and caused serious damage to shrimp farms in the world. Detection of white spot syndrome virus (WSSV) in shrimps is very important for management of shrimp farms. WSSV test kit, named Shrimple is a management tool, based on immunoassay. It can be used for shrimp aquaculture to assess the level of WSSV infection in larva and shrimp during breeding. Shrimple is a lateral flow, one step, fast and easy to use test-kit. The results are read visually without any instruments. The test system employs unique monoclonal antibodies to selectively identify WSSV in shrimps, with a high degree of sensitivity.

TEST PRINCIPLE

Shrimple is based on the principle of a sandwich immunoassay. The test device consists of a membrane strip and plastic case. A colored monoclonal rat anti-WSSV antibody colloid gold conjugate (antibody - colloid gold conjugate) pad is placed next to the sample pad region on the membrane strip. The membrane strip is also precoated with a different monoclonal rat anti-WSSV antibody on the test (T) zone and anti-rat ImmnoglobulinG (IgG) antibody on the control (C) zone.

In the absence of WSSV in the shrimp, the antibody-colloid gold conjugate moves with the sample, by capillary action along the membrane through the T zone until it reaches the C zone. At this point, antibody-colloid gold conjugate reacts with the pre-coated anti-rat IgG antibody. This produces a visible pink colored band. When only one pink colored band appears on the C zone, it When the WSSV is present in means negative. the shrimp, the antibody-colloid gold conjugate reacts with WSSV to produce conjugate-WSSV complex and moves until it reaches the T zone. At this point, the complex reacts with the pre-coated different monoclonal rat anti-WSSV antibody to produce a visible pink colored band. Excess antibody-colloid gold conjugate passes through the T zone until the C zone. At this point, the excess antibody-colloid gold conjugate reacts with the pre-coated anti-rat IgG antibody to produce a visible pink colored band. When the pink colored band appears in both T zone and C zone, it means positive.

ILLUSTRATION OF MECHANISM



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KITS CONTENT

Each kit consists of following parts:

- Test Device
- Micro tube with 0.6ml buffer liquid (20mM, TBS)
- Stick
- Dropper

SPECIMEN COLLECTION

Shrimps that look sick or unhealthy should be used. No special pre-treatment is necessary. The specimen collection may be stored up to 2 days at refrigerated condition (2 to -8°C). If it is necessary to store the specimen more than 3 days, it should be stored under frozen condition(-20°C or below).

Note: Do not use shrimps stored in alcohol as they adversely affect the test results.

TEST PROCEDURE

Please refer to the illustration of test on the last page.

 Count the number of dead shrimps with each culture pond every day. When the number is more than usual or a lot of shrimps are floating on the surface of the water, pick up some of the shrimps that look sick or unhealthy. 2. Wash your hands well before starting the test.

The shrimps to be tested should be washed with clean water. Take the sample quantity as mentioned in the table below and put into the buffer tube provided.

3. The number of testing sample required of one micro tube is as follows:

Shrimp Size	Testing Sample	No. Of Shrimp Required
PL7	Whole body	20
PL10 or less	Whole body	10
PL20 or less	Whole body	5
0.05g or less	Whole body	2
0.1g or less	Whole body	1
0.5g or less	Whole body without head & carapace	1
2g or less	All legs	1
3g or less	All swimming legs	1
5g or less	Five swimming legs	1
10g or less	Three swimming legs	1
20g or less	Two swimming legs	1
Over 20g	One swimming leg	1

Note: Adjust sample volume to about 0.1g each 1 test.

Note: It is recommended that max 10 tests per 1 time be conducted with unhealthy or dead shrimps in the nursery over once a week even if the nursery has a well maintained.

4. Pulverize the testing samples in the micro tube with the stick provided in the kit. This should be done about ten times. Then settle the solution for five minutes and take the supernatant with the dropper and put 4 drops into the round hole of the Shrimple device.

Wait for 15 minutes and read the results. See following for interpretation of the results.

INTERPRETATION OF RESULTS

Negative: Only one pink colored band appears in the C zone. (In rare case, there is appearance of weak and non-pink colored band in the T zone, according to environmental influence. It means Negative result).

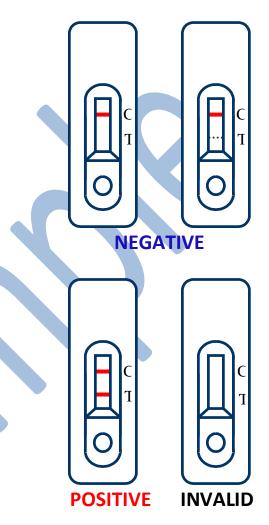
Positive: In addition to one pink colored band in the C zone, a distinct pink colored band will also appear in the T zone.

Invalid: No pink colored band appears in the C zone. An invalid result may be due to improper testing procedures or deterioration of the kit components. Repeat the assay sequence using a new device.

Note: Read the test result within 15 minutes as the band becomes indistinguishable with its color getting thinner with time going by

Note: When at least one test shows positive, assume that many of the shrimps in the culture pond are infected. Such positive result means the dangerous possibility that a mass death will occur in the following one week is high. When up to ten tests show negative, you may assume that

The shrimps in the nursery are not infected or that there will be less likelihood of mass death.



STORAGE AND STABILITY

The kit can be stored at room temperature in the sealed pouch to the date of expiration. The kit should be kept away from direct sun light, moisture and heat. Expiration date is described on the product package

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Shrimple

White Spot Syndrome Virus Test Kit

PRECAUTIONS

- 1. For detecting WSSV in the larva and shrimp only.
- 2. The test device is used once and should not be reused.
- 3. The buffer liquid in the micro tube should be used for virus testing purpose only. Avoid contact with skin or eyes.
- 4. Do not use test kit beyond the expiry date.
- 5. Discard the used kit in a safe place

IMPORTANT: Technical information contained herein is furnished without charge or obligations, and is given and accepted at recipient's sole risk. We are not responsible for any data and/or results with this kit because the conditions of use kit may vary and are beyond our control.

TEST ILLUSTRATION

