

A rapid immunochromatographic strip test for the detection of *Vibrio parahaemolyticus* toxin B that cause Early Mortality Syndrome (EMS) in shrimp

Early Mortality Syndrome (EMS) or acute hepatopancreatic necrosis disease (AHPND) is a highly virulent disease that causes mass mortality in farmed penaeid shrimps worldwide. The causative agent of EMS/AHPND was identified as a unique isolate of *Vibrio parahaemolyticus* (VP_{AHPND}) containing a 69–70 kb plasmid encoding toxins (ToxA and ToxB) homologous to but distinct from *Photobacterium* insect related (pir) toxins. Recently, monoclonal antibodies (MAb) specific to ToxA and ToxB were generated by a research team at Department of Biology, Faculty of Science, Srinakharinwirot University. These MAbs can be used to detect toxins from VP_{AHPND} isolate by dot blotting and the MAb specific to ToxB was also used for quantitative analysis of the toxin in culture by indirect ELISA. These MAbs were further developed into immunochromatographic strip tests for the detection of ToxB of VP_{AHPND}. The strip test has the advantages of speed, as the result can be obtained within 10-15 min, and simplicity, as laboratory equipment and specialized skills are not required. Therefore, strip tests can be used by shrimp farmers for the pond-side monitoring of VP_{AHPND} infection.

Test Procedure

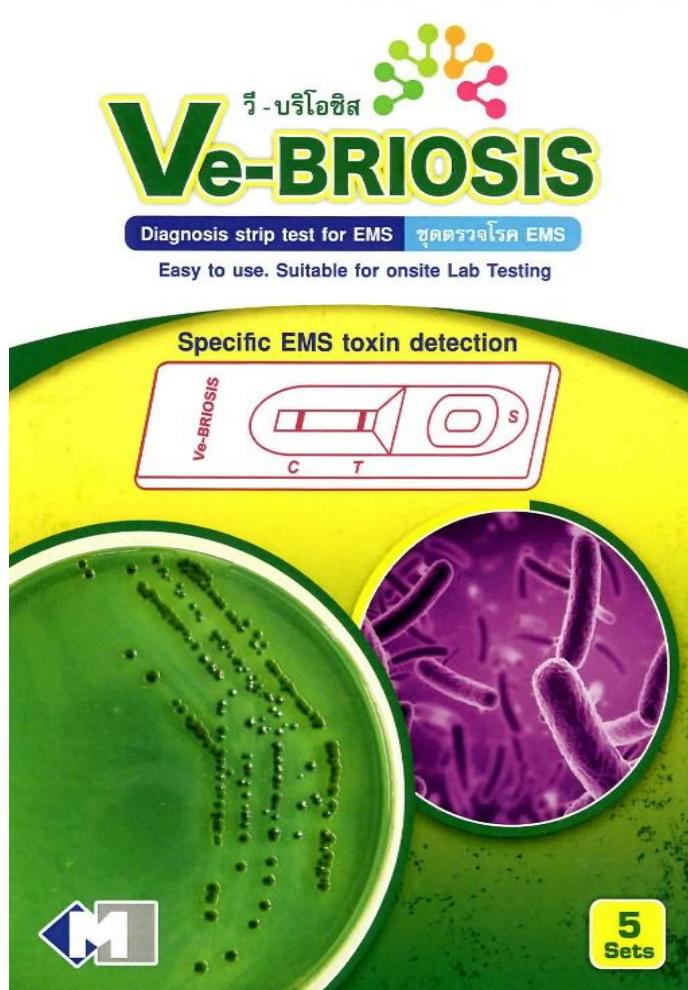
1. The target tissues from hepatopancreas, gut, shrimp larvae, artemia, water in rearing tank or other risk factor of EMS infection were used to culture the bacteria on agar plate (TSBS or CHROM agar)
2. The colony from agar plate was taken and put in application buffer, and grind with a plastic pestle.
3. The dropper was used to withdraw supernatant of 100-150 μ l or 5-6 drops and slowly drop supernatant into the sample well. The results can be observed within 5-15 minutes.

Result

The two reddish-purple bands found on the test line (T) and control line (C) indicate that the sample tested is infected with EMS. If a band is only found on the C line, it indicates the negative result meaning that there is no infection or the shrimp may be infected with the AHPND bacteria in a lower amount than the sensitivity of the test kit.

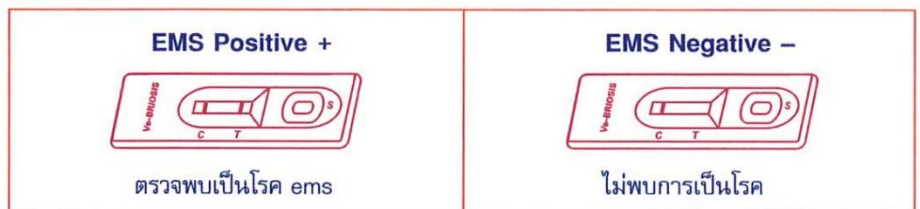
**If a reddish-purple band in the C line does not appear, the test kit has a problem or may have a sample preparation error.

***This test kit is only a screening or confirmation of the EMS infection. As a result of the negative test, the test should be confirmed by PCR or higher sensitivity method.



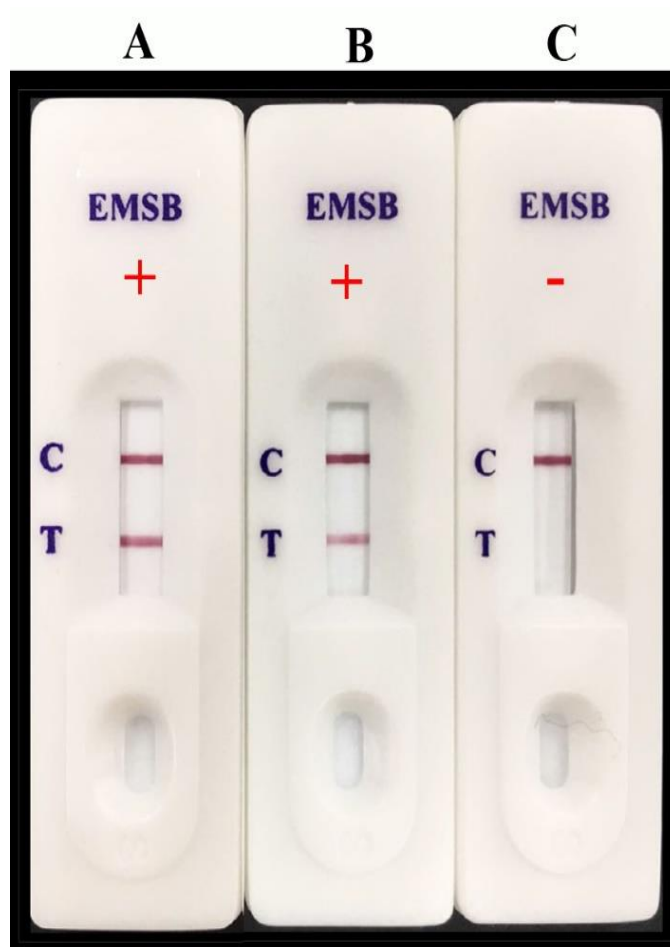
Commercially available: Early Mortality Syndrome (EMS) test kit.
(Distributed by Marine Leader Co. Ltd.)

วิธีอ่านผล | Reading the result



The reddish purple bands on the Control (C) together with EMS Toxin Line (T) indicates EMS Toxin infection respectively. If a band only occurs at the C line, indicates the negative result or the infection is too low to be detected by the test kit.

Reading the result
(Marine Leader Co. Ltd.)



Test strip results. The colonies of bacteria cultured on TSA from three isolates of *Vibrio parahaemolyticus*, A) VP_{AHPND} from China, B) VP_{AHPND} from Thailand and C) VP_{non-AHPND}, were transferred into application buffer and applied to the test strip. T = test line; C = control line. + = Positive; - = Negative