Title : Development of Recombinase Polymerase Amplification Combined with Lateral Flow Assay for Rapid Identification of *Flavobacterium covae* and *Flavobacterium oreochromis* in Tilapia and Asian Seabass

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ABSTRACT

Columnaris disease caused by a group of *Flavobacterium* spp. can lead to significant economic losses in aquaculture industry. *Flavobacterium covae* and *F. oreochromis* are two predominant pathogens affecting tilapia and Asian seabass farming in Thailand. Although the standard PCR is effectively used for early detection of the disease, it remains limited by time consumption, high equipment costs, and the requirement of skilled users to perform the tests. Therefore, this study aims to develop a duplex recombinase polymerase amplification (RPA) technique in combination with a lateral flow assay (LFA) to accelerate the early monitoring of *F. covae* and *F. oreochromis* in tilapia and Asian seabass. The study involved the design of primers specific for *F. covae* (*aroB* gene) and *F. oreochromis* (*odE1* gene), optimization of RPA conditions, specificity, and sensitivity tests. Our results showed that the optimal condition for the duplex RPA was an incubation at 39°C for 35 min, followed by 20 min of LFA. The developed RPA-LFA was highly specific for both *F. covae* and *F. oreochromis* without cross-reactivity with other fish pathogens. The RPA-LFA was also highly sensitive with a limit of detection (LoD) as low as

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0.64 pg/µL and 0.4 ng/µL for *F. covae* and *F. oreochromis*, respectively. Additionally, the developed RPA-LFA was challenged with real samples collected from fish farms with the records of the disease outbreak. From a total of 25 gill samples from infected fish, the assay detected *F. covae* in 6 samples, *F. oreochromis* in 10 samples, and both bacterial species in 2 samples. These results showed a consistency of 96% with the PCR technique. It is anticipated that the developed duplex RPA-LFA technique could become an invaluable bioanalytical alternative for controlling the outbreak of columnaris disease, especially for onsite applications and resource-limited laboratories, and subsequently supporting aquaculture industry of Thailand.

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