**Supplemental Table 4.** Summary of qPCR testing for the detection and quantification of pathogens in tilapia tissue samples

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Pathogen** | **Method** | **Target** | **Reaction** | **Cycling conditions** | **qPCR performance** | **Reference** |
| **Tilapia lake virus (TiLV)** | Hydrolysis probe RT-qPCR  | TiLV Segment9 | A 20 µL qPCR reaction contained the 200 ng template, 450 nM of each primer, 150 nM probe and 1X qScript One-Step RT-qPCR Kit (Quanta bio, Cat no#95134-500) | Reverse transcription 50°C for 10 min, 95°C for 1 min and 40 cycles of 95°C for 10 s and 58°C for 30 s | Y = -3.476 X + 42.295R2 = 0.998E = 94.0% | (Taengphu et al., 2022) |
| **Infectious spleen and kidney necrosis virus (ISKNV)** | Hydrolysis probe qPCR  | ISKNV major capsid protein gene (MCP) | A 20 µL qPCR reaction contained the 200 ng template, 900 nM of each primer, 250 nM probe and 1X iTaq Universal Probes supermix (BioRAD, Cat no#172-5131) | 95°C for 10 min and 40 cycles of 95°C for 15 s and 64°C for 1 min  | Y = -3.480X + 41.674R2 = 0.997E = 93.8% | (Kawato et al., 2020) |
| ***Francisella orientalis* (*FnO*)** | SYBR qPCR  | *F. orientalis* hypothetical protein (HP) gene | A 20 µL qPCR reaction contained the 200 ng template, 200 nM of each primer and 1X KAPA SYBR FAST qPCR Master Mix(KapaBiosystems, Cat no#KK4600) | 95°C for 3 min and 40 cycles of 95°C for 30 s and 60°C for 30 s followed by melt curve analysis | Y = -3.563X + 39.873R2 = 0.999E = 90.8% | This study |
| ***Streptococcus agalactiae* (SAG)** | Hydrolysis probe qPCR | *S. agalactiae* groEL gene | A 20 µL qPCR reaction contained the 300 ng template, 900 nM of each primer, 250 nM probe and 1X iTaq Universal Probes supermix (BioRAD, Cat no#172-5131) | 95°C for 2 min and 40 cycles of 95°C for 5 s and 60°C for 30 s | Y = -3.581X + 43.424R2 = 0.991E = 90.2% | Modified from(Leigh et al., 2018) |

**References**

Kawato Y, Mohr PG, Crane MSJ, Williams LM, Neave MJ, Cummins DM, Dearnley M, Crameri S, Holmes C, Hoad J, Moody NJG. 2020. Isolation and characterisation of an ISKNV-genotype megalocytivirus from imported angelfish Pterophyllum scalare. *Diseases of Aquatic Organisms* 140:129–141. DOI: 10.3354/dao03499.

Leigh WJ, Zadoks RN, Jaglarz A, Costa JZ, Foster G, Thompson KD. 2018. Evaluation of PCR primers targeting the groEL gene for the specific detection of Streptococcus agalactiae in the context of aquaculture. *Journal of Applied Microbiology* 125:666–674. DOI: 10.1111/jam.13925.

Taengphu S, Kayansamruaj P, Kawato Y, Delamare-Deboutteville J, Mohan CV, Dong HT, Senapin S. 2022. Concentration and quantification of Tilapia tilapinevirus from water using a simple iron flocculation coupled with probe-based RT-qPCR. *PeerJ* 10:e13157. DOI: 10.7717/peerj.13157.