**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 + 42.295  R2 = 0.998  E = 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 + 41.674  R2 = 0.997  E = 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 + 39.873  R2 = 0.999  E = 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 + 43.424  R2 = 0.991  E = 90.2% | Modified from  (Leigh et al., 2018) |

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