Optimization of a qPCR procedure for detection of IHHNV in shrimp

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Background

- Decapod penstyldensovirus-1 (PstDV-1)
  - Formaly infectious hepodermal and hematopoietic necorsis virus (IHHNV)
  - Single stranded DNA virus (22nm)
  - Pathogenic for a variety of decapods (*L. vannamei, P. stylirostris, P. monodon*,…)
  - Transmission
    - Horizontally: water, cannibalism
    - Vertically: through breeding

Background

- Acute: Up till 100% mortality
- Chronic: *L. vannamei*: runt-deformities syndrome (RDS)
  - Reduced growth
  - Irregular growth
  - Cuticular deformities
  - Reduced hatching and larval survival
  - Impact of stress event

- No effective vaccination
- Mainly focus on genetic selection

- Detection of the virus of critical importance
  - General symptoms
  - Chronic symptoms

- Use of (q)PCR to determin presence and amount of viral genoms as main monitoring strategy

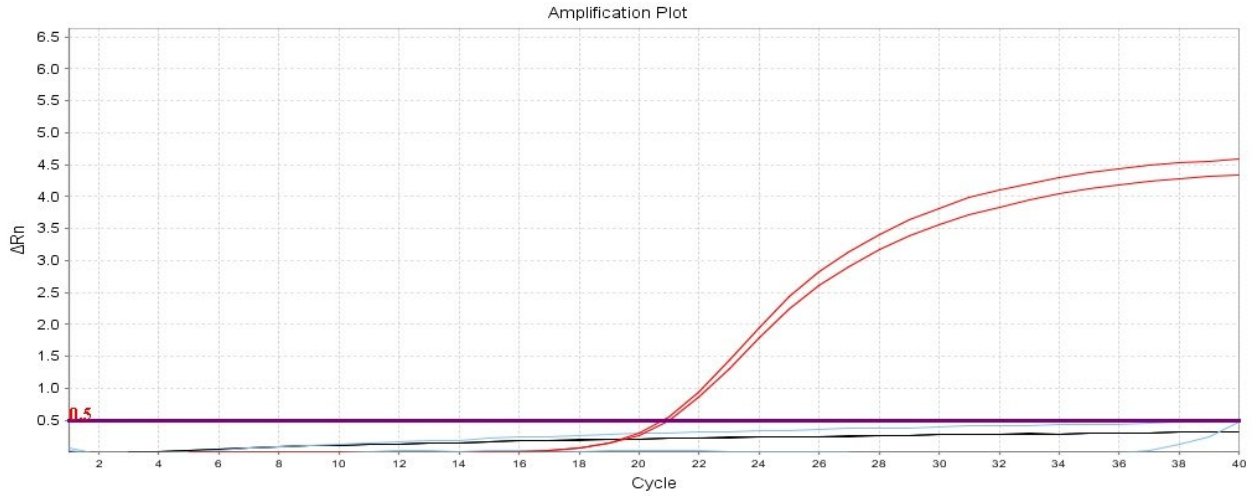
Jagadeesan et al., 2019

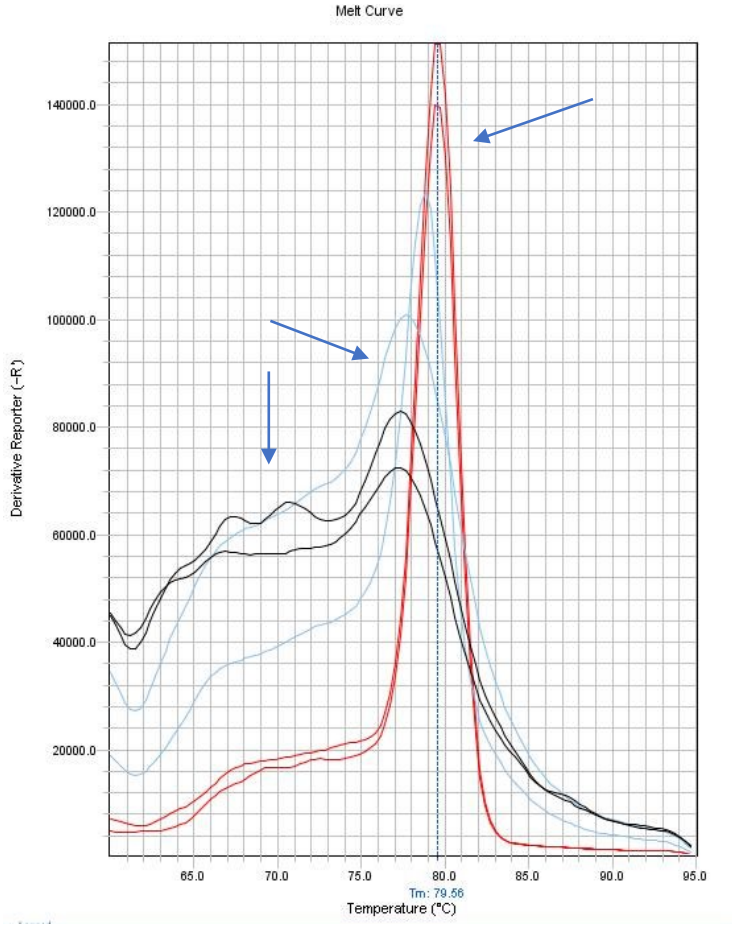
IMAQUA

- Diagnostic (q)PCRs for:
  - PstDV-1
  - EMS
  - WSSV
  - IMNV
- qPCRs available for pathogens and immune related genes
- SYBR green based
  - Optimization needed from TaqMan protocols in literature

IMAQUA PstDV-1

- PstDV-1 qPCR showed several points of improvement
  - Several cases of uncertain results due to a amplification peak with melting temperature (MT) close to target MT
  - Improve sensitivity

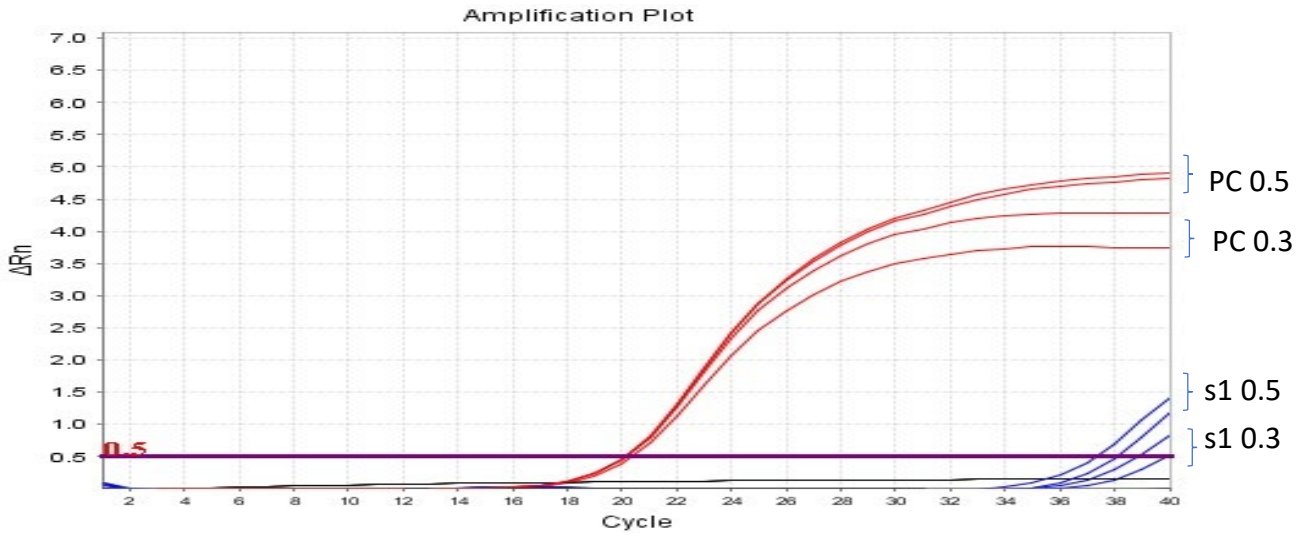




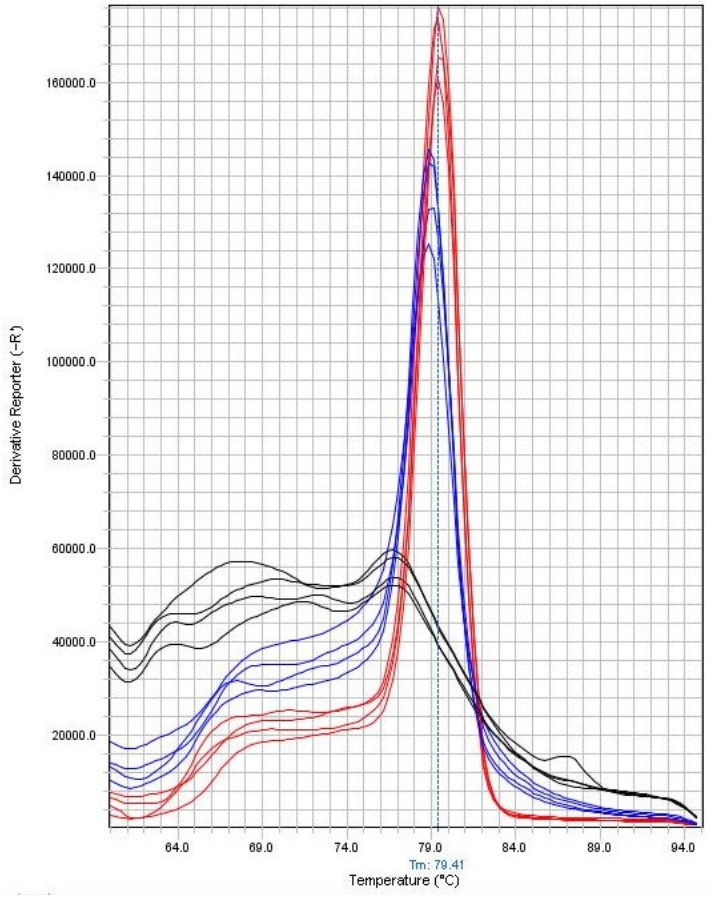
Sample	Ct value	Melting temperature
PC	21.90227	79.56414
PC	22.07962	79.56414
S1	Undetermined	78,82011
S1	Undetermined	77,62524

qPCR PstDV-1

- Amplification outside correct melting temperature:
  - Excess primer?
  - 2 different primer concentrations tested: 0.3 and 0.5 μ M

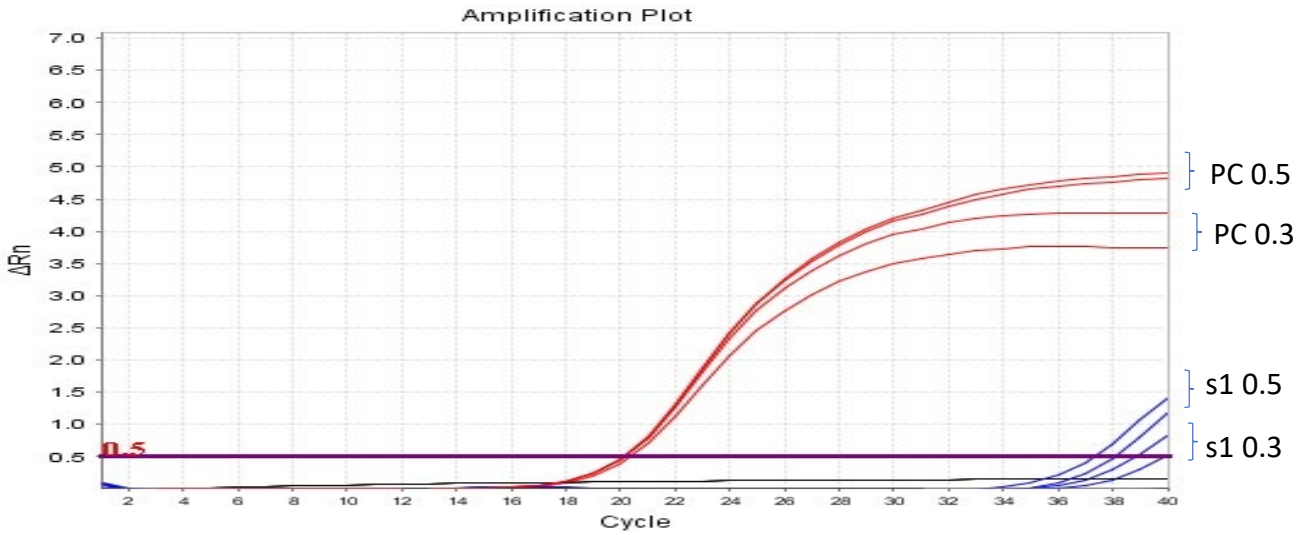


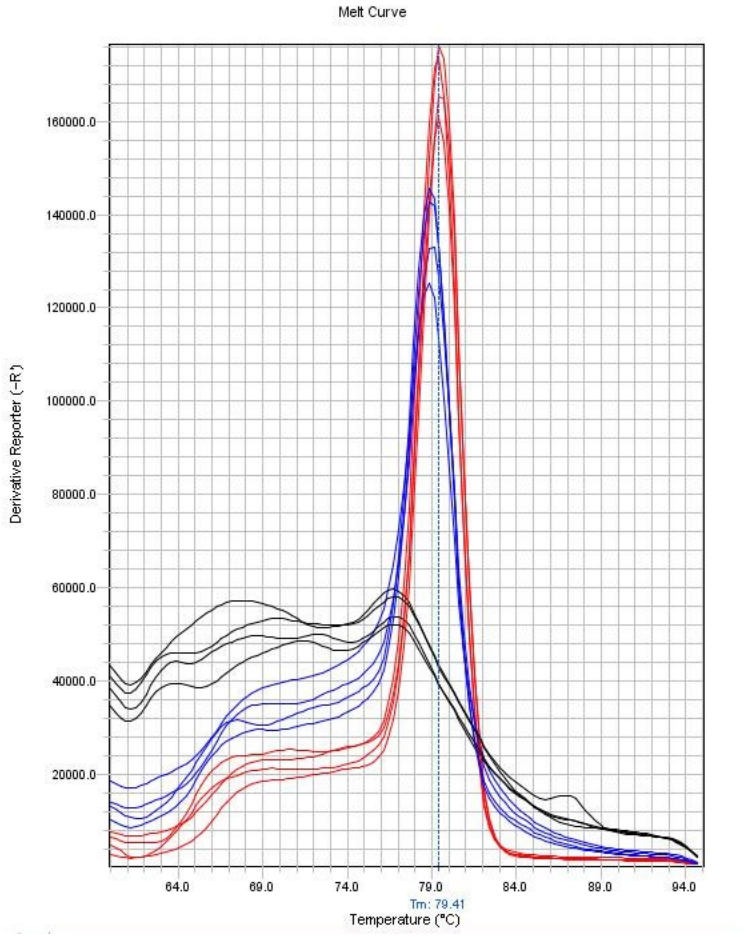
Sample	Ct value	Melting temperature
PC 0.5	20.1411438 / 20.10007668	79.5640335 / 79.41468811
PC 0.3	20.1176815 / 20.34446144	79.2653427 / 79.41468811
S1 0.5	38.08307266 / 37.31864929	78.8195572 / 78.96802521
S1 0.3	39.90710068 / 38.87400818	78.8195572 / 78.96802521



qPCR PstDV-1

- Amplification outside correct melting temperature:
  - Excess primer?
  - 2 different primer concentrations tested

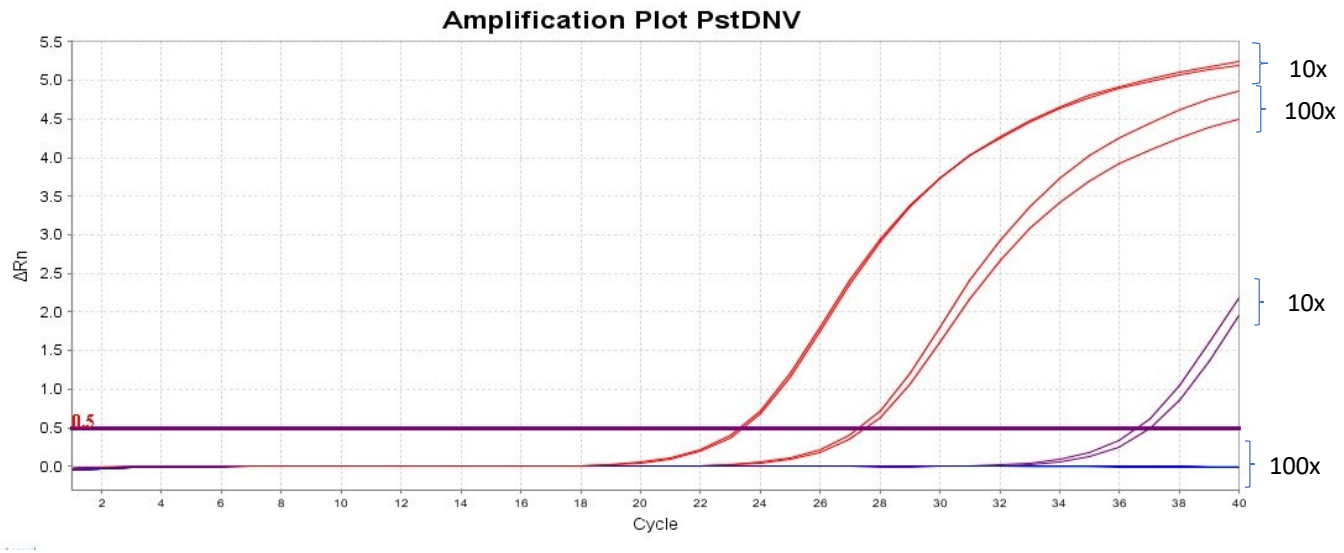




=> Not a big impact on results

qPCR PstDV-1

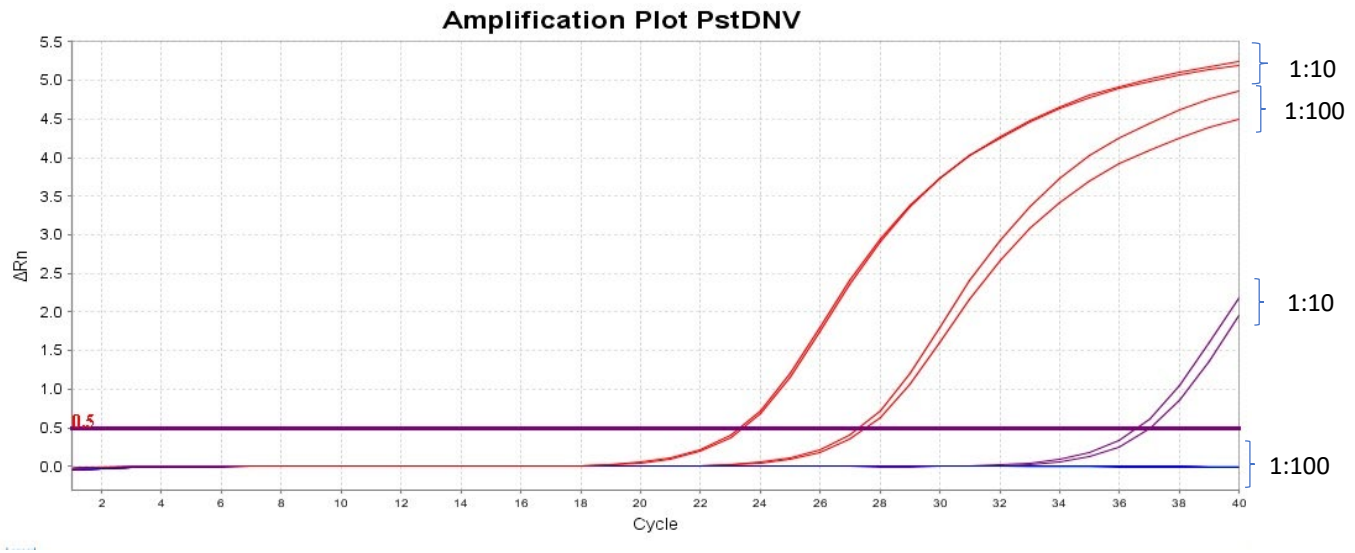
- Amplification outside correct melting temperature:
  - Inhibitors in shrimp tissue
    - Diluting samples
      - 1:10
      - 1:100

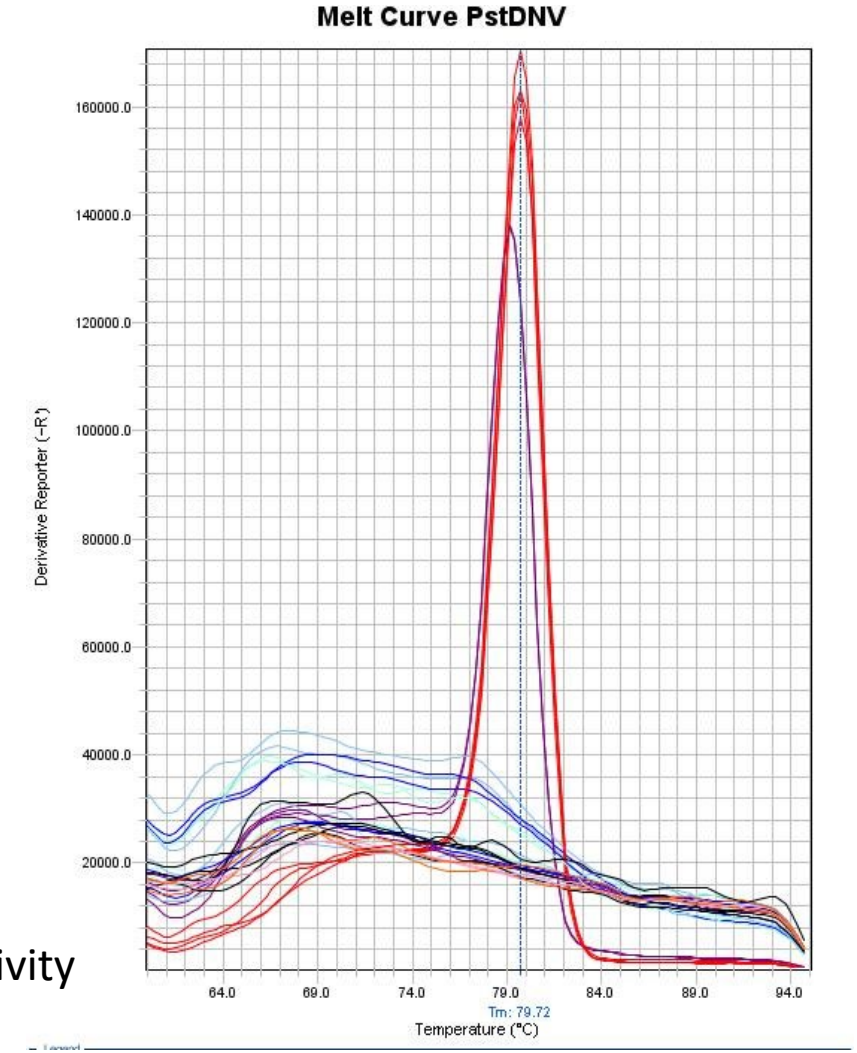


Sample	Ct value	Melting temperature
PC 1:10	23.32539368	79.716507
PC 1:10	23.43229485	79.716507
PC 1:100	27.31265068	79.716507
PC 1:100	27.55193329	79.716507
S1 1:10	36.99858093	79.1175156
S1 1:10	36.60836792	79.1166992
S1 1:100	Undetermined	67.3175964
S1 1:100	Undetermined	67.9149933

qPCR PstDV-1

- Amplification outside correct melting temperature:
  - Inhibitors in shrimp tissue
    - Diluting samples
      - 1:10
      - 1:100

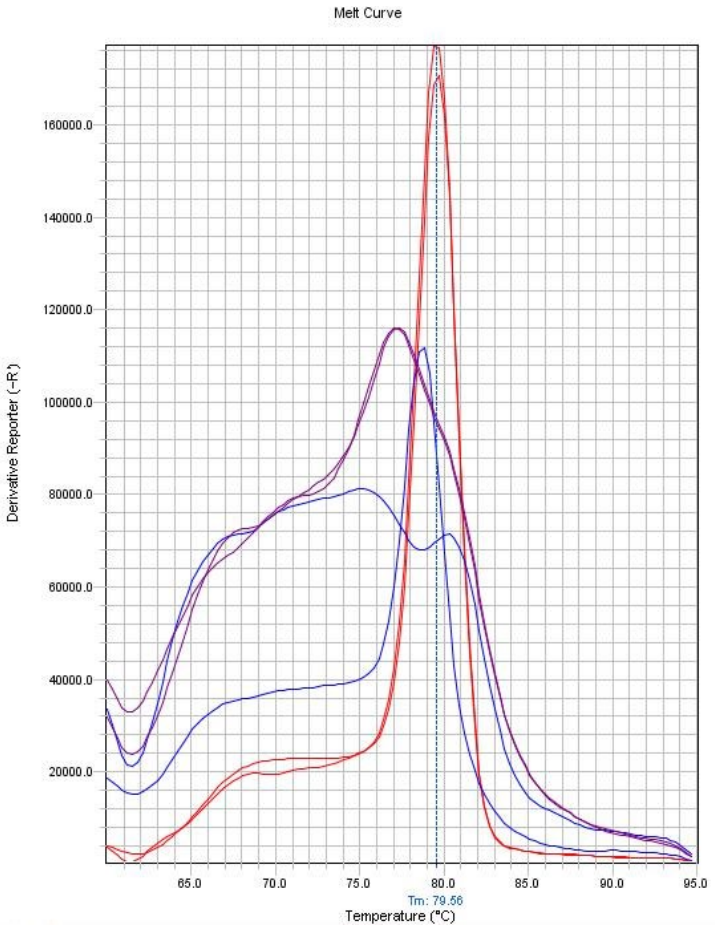


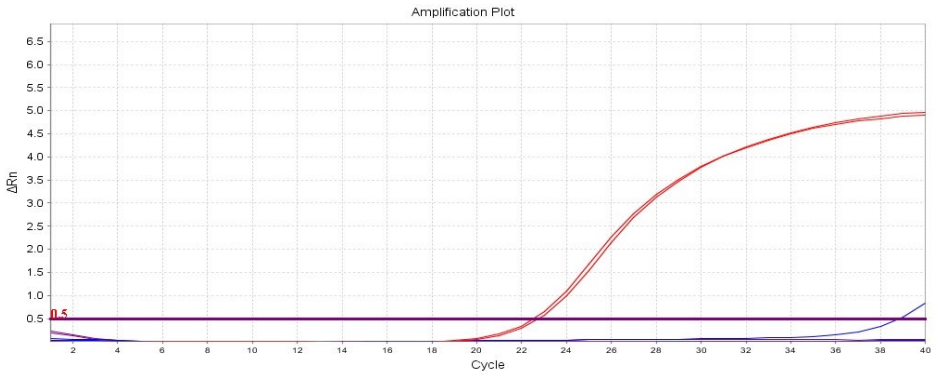


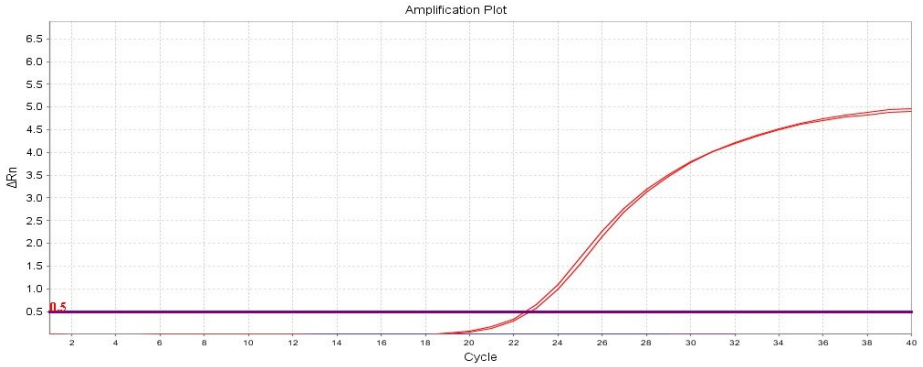
⇒ Diluting 1:10 lowered inhibition effects with still high enough sensitivity

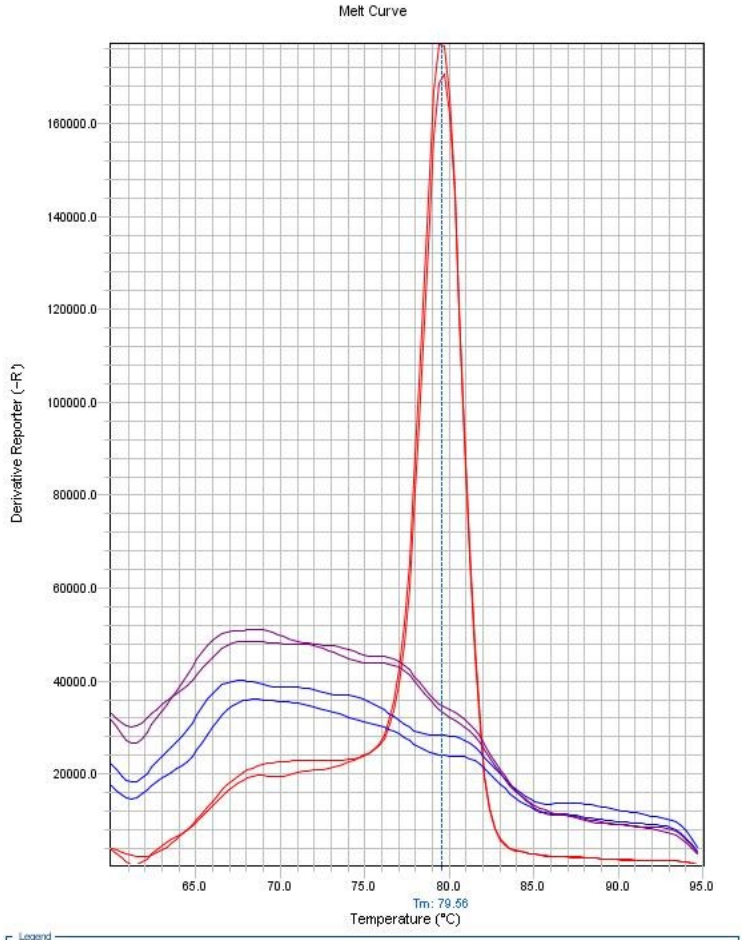
⇒ Diluting 1:100 lowered sensitivity too much

qPCR PstDV-1: example 2







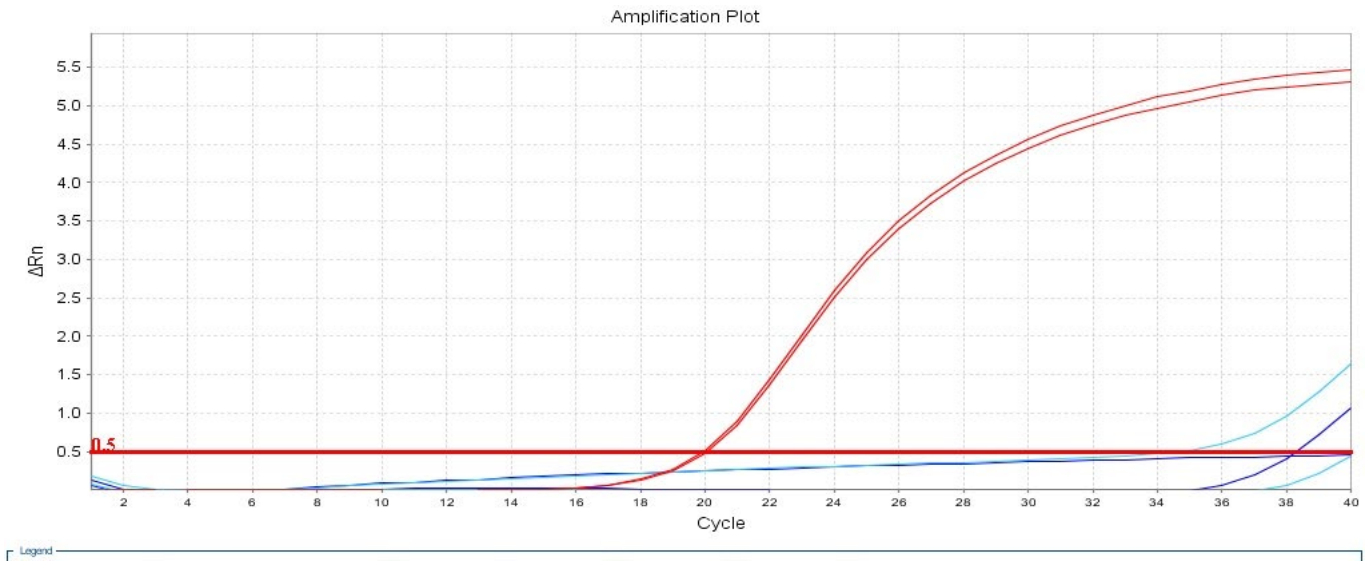


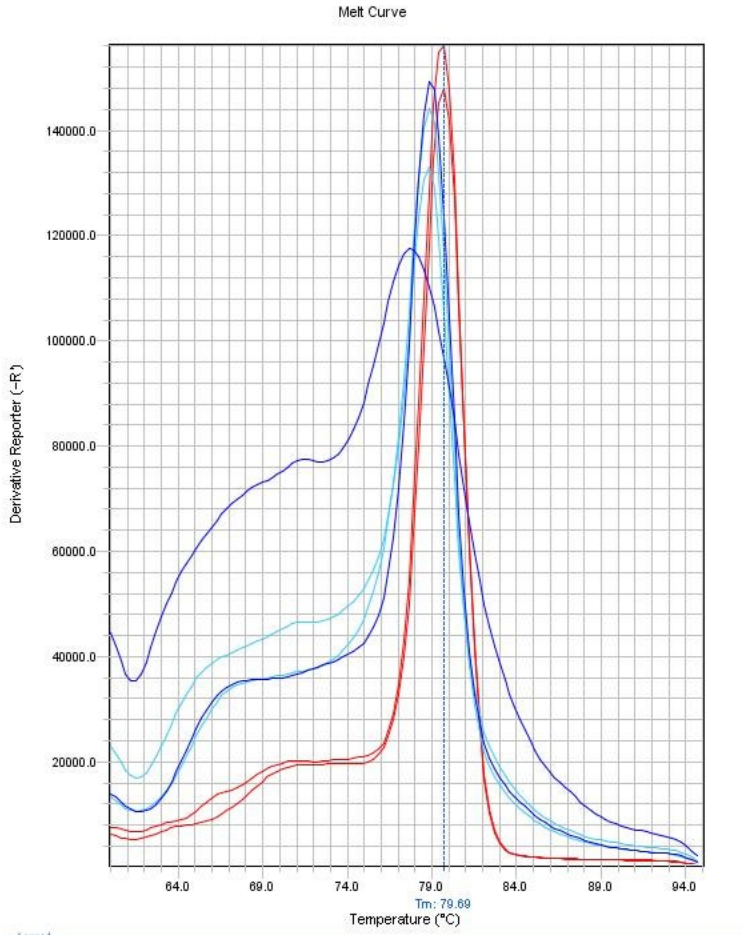
qPCR PstDV-1

- Ct-values undetermined but correct melting temperature
  - PCR byproduct?
  - PstDV-1 amounts too low?

- Stress test:
  - Cold temperature shock for 24h
  - Normal husbandry conditions 48h
  - => induction of viral replication

Stress test





Sample	Ct value	Melting temperature
PC	29.95577	79.71076
PC	21.06002	79.56143
S1	39.53812	78.96996
S2	37.74538	78.82062
S3	35.6974	79.26678

=> Batch with undetermined Ct-values became determined

Conclusion

- PstDV-1 qPCR protocol was adjusted with the following steps:
  - Samples are 1:10 diluted
  - Inclusion of stress test for a susceptive PstDV-1 shrimp group in chronic infection with sublethal or subtle symptoms.

- Although qPCR was already developed for PstDV-1, continuous optimization is necessary

- This allows further improvement of the sensitivity and certainty in our routine pathogen (PstDV-1) detection

THANK YOU FOR YOUR ATTENTION

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