White spot disease (WSD)


*image from DAF Biosecurity Qld "WSD overview"

- Disease of crustaceans
  - Highly contagious
  - Caused by white spot syndrome virus (WSSV)
- Major health problem for prawn industry
  - Infected ponds 80-90% mortality
  - No treatment or vaccine
- 2016 Logan River outbreak
  - Significant loss of livestock and economic cost
  - Exhaustive biosecurity response
  - Restricted to SE Qld but ongoing surveillance

WSSV diagnosis

- Field sampling & processing
  - Labour intensive
  - $$$
- Government Laboratory testing
  - Specialist training
  - $$$
  - Results 24-72 hrs once sample is received
  - Confirmation of positives required at 2nd lab

Current tests for WSSV

- In Australia:
  - Taqman Real Time PCR (qPCR) ➡ Gold Standard
  - Conventional PCR
  - Sequencing

  } confirmation
- Other methods recommended by OIE:
  - Loop-mediated isothermal amplification (LAMP)
- DNA detection
  - Capacity to detect low viral load

Point of Care detection

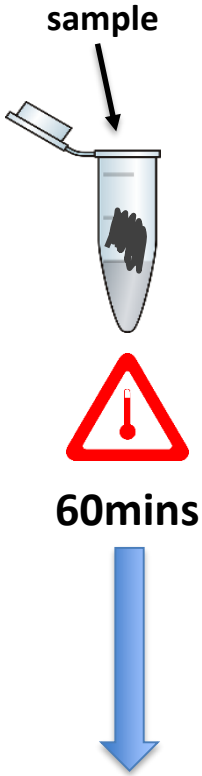
- Important tool in the future
- Simple › fast › sensitive › accurate
- Mainly detect virus proteins
  - Rapid and easy to use
  - Useful for confirming infections
  - BQ evaluating Shrimple®
- What about DNA detection?
  - No stick test available
  - IQ Plus WSSV Kit with POCKIT System

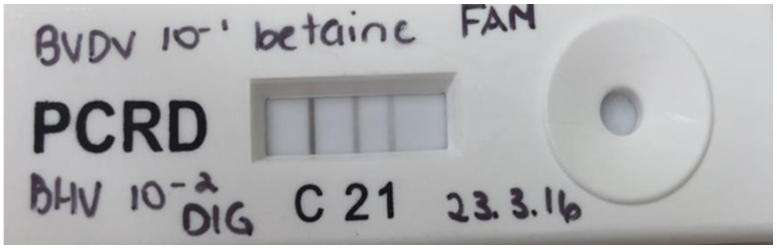
*image from https://www.fkkasei.co.jp/


*image from http://iq2000kit.com/

Our lab's POC assay for BRD

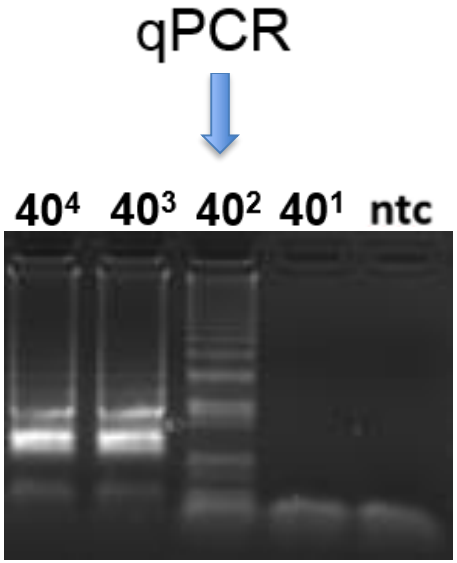
- LAMP based method
- Pathogen specific
- Limit of detection = qPCR
- 2hrs vs 24-72hrs
- Lateral flow device or stick test
- DNA or crude sample
- Simple readout

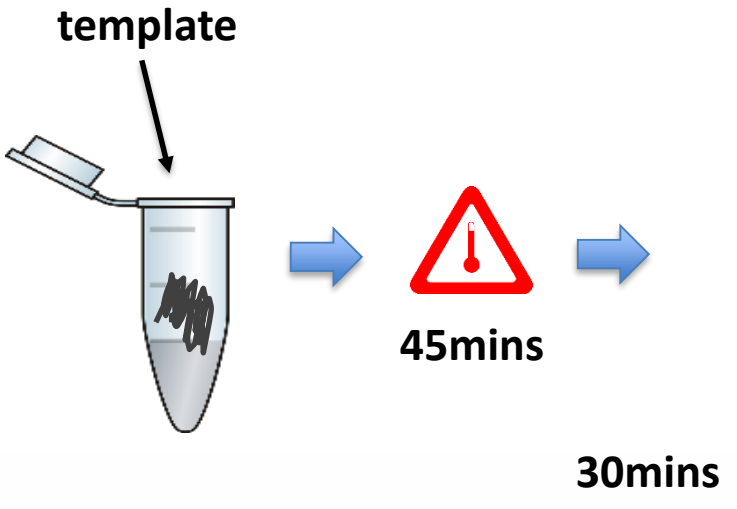


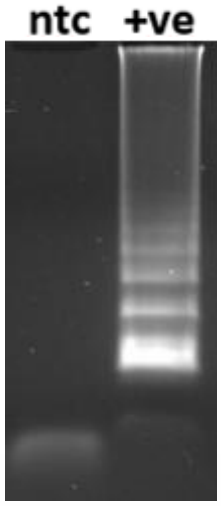


Adapt LAMP assay for WSD?

- YES!
  - Conserved WSSV gene target
  - Synthetic positive control (370bp)
- Limit of detection
  - Similar to qPCR
- Detection on lateral flow device







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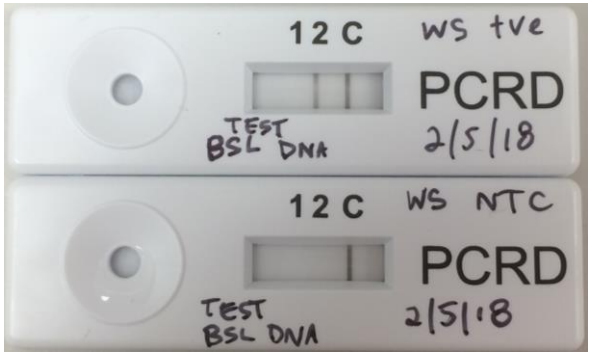


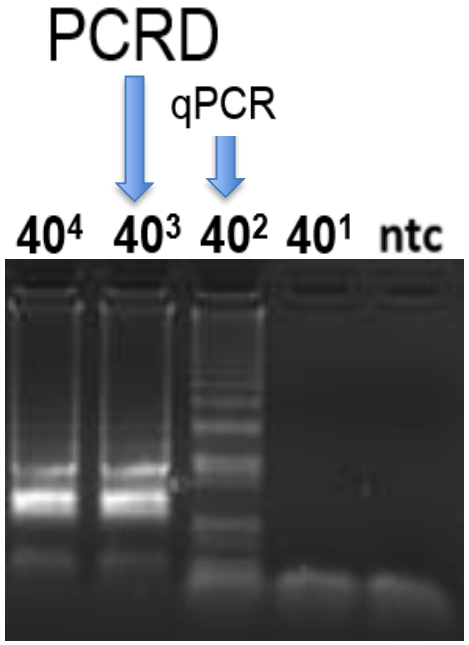
10mins

Test on WSSV samples (BSL)

- 100 samples tested in LAMP assay
  - WSSV status undisclosed prior
  - Equal positive vs negative requested
- 88% agreement with qPCR ☹
  - 6 false negatives
  - 6 false positives
- Why???
  - Could samples be mixed up? ➡ qPCR says no ✓
  - Could PCRD be less sensitive?
  - Are there non-specific products?

} Troubleshoot

Sensitivity of LAMP



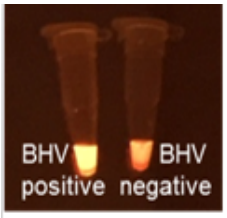
- PCRD limit of detection = 40^3
- All 6 PCRD false negative samples:
  - qPCR positive with cT ≥ 30
  - suggesting lower viral load
  - LAMP positive on gel
- PCRD for LAMP detection may not suitable for detection of low level infection

Specificity of LAMP

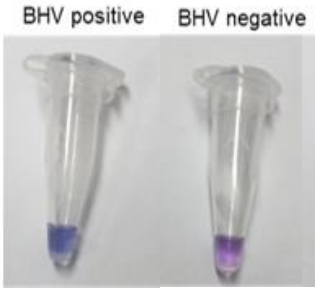
- 94% agreement with qPCR
- All 6 PCRD false positive samples:
  - Negative by qPCR
  - But LAMP positive on gel, sometimes!
  - Non-specific products ✓
- Increase reaction specificity
  - No more false positives
  - 100% agreement

*image from http://1.bp.blogspot.com

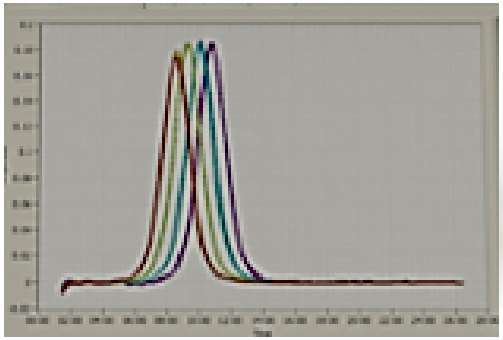
Alternative detection methods



1. Visual methods:
   - Fluorescence, colour change, turbidity
   - Tried for BRD LAMP
   - OK, but too subjective



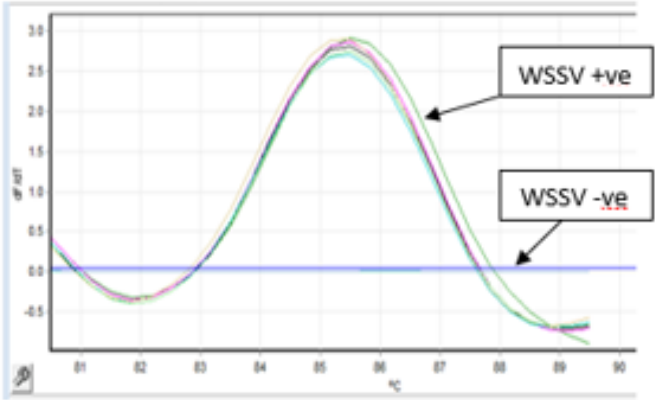
2. Fluorescent melt analysis
   - Same assay reaction, just add fluorescent dye
   - Needs real-time PCR machine
   - Portable field unit available
   - No open tube
   - Easy to interpret



*Images from http://www.optigene.co.uk/

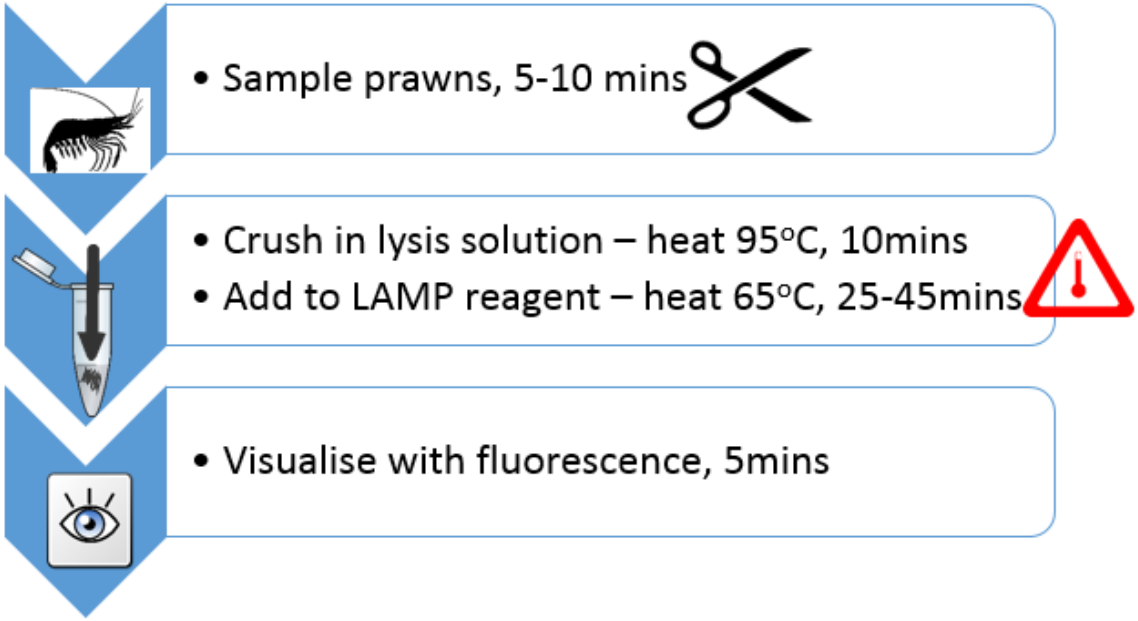
Fluorescent WSSV LAMP

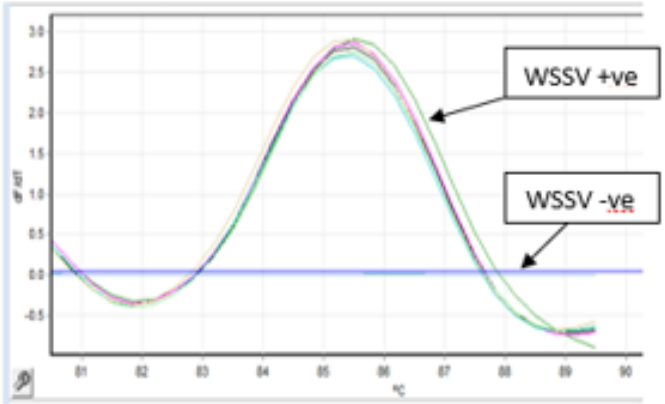
- Limit of detection better than qPCR
- Specificity & accuracy 100%
- All samples correct ✓
- Works on crude tissue!
  - No DNA Extraction
  - Short pre-treatment
  - Trialled on 10 frozen BSL samples ✓
  - No assay inhibition
- Results <80mins



LAMP-WSD test Workflow

(~ total time <45-70mins)





Where are we at?

- Developed WSSV LAMP assay
  - Relatively simple
  - Sensitive, specific & accurate
  - Can test crude samples with minimal preparation
  - Comparable to qPCR
  - Fast workflow with a result possible in < 1.5hrs
  - POC detection methods available

Where to next?

- Assay validation in large sample study
- Lab-ready in December for BSL
- Portable device testing
- Test assay on other animals
  - WSSV hosts or carriers
- Future research
  - Develop in-house stick test
  - Dehydrated assay mix
  - Field testing
- BQ approval for POC testing

Thank you!

- Dr Rebecca Ambrose, DAF
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