White spot disease (WSD)

• 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 2\textsuperscript{nd} lab
Current tests for WSSV

• In Australia:
  – Taqman Real Time PCR (qPCR) **Gold Standard**
  – Conventional PCR
  – Sequencing

• 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
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 $cT \geq 30$
  - 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

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LAMP-WSD test Workflow
(≈ total time <45-70 mins)

- Sample prawns, 5-10 mins
- Crush in lysis solution – heat 95°C, 10mins
- Add to LAMP reagent – heat 65°C, 25-45mins
- Visualise with fluorescence, 5mins

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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!

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