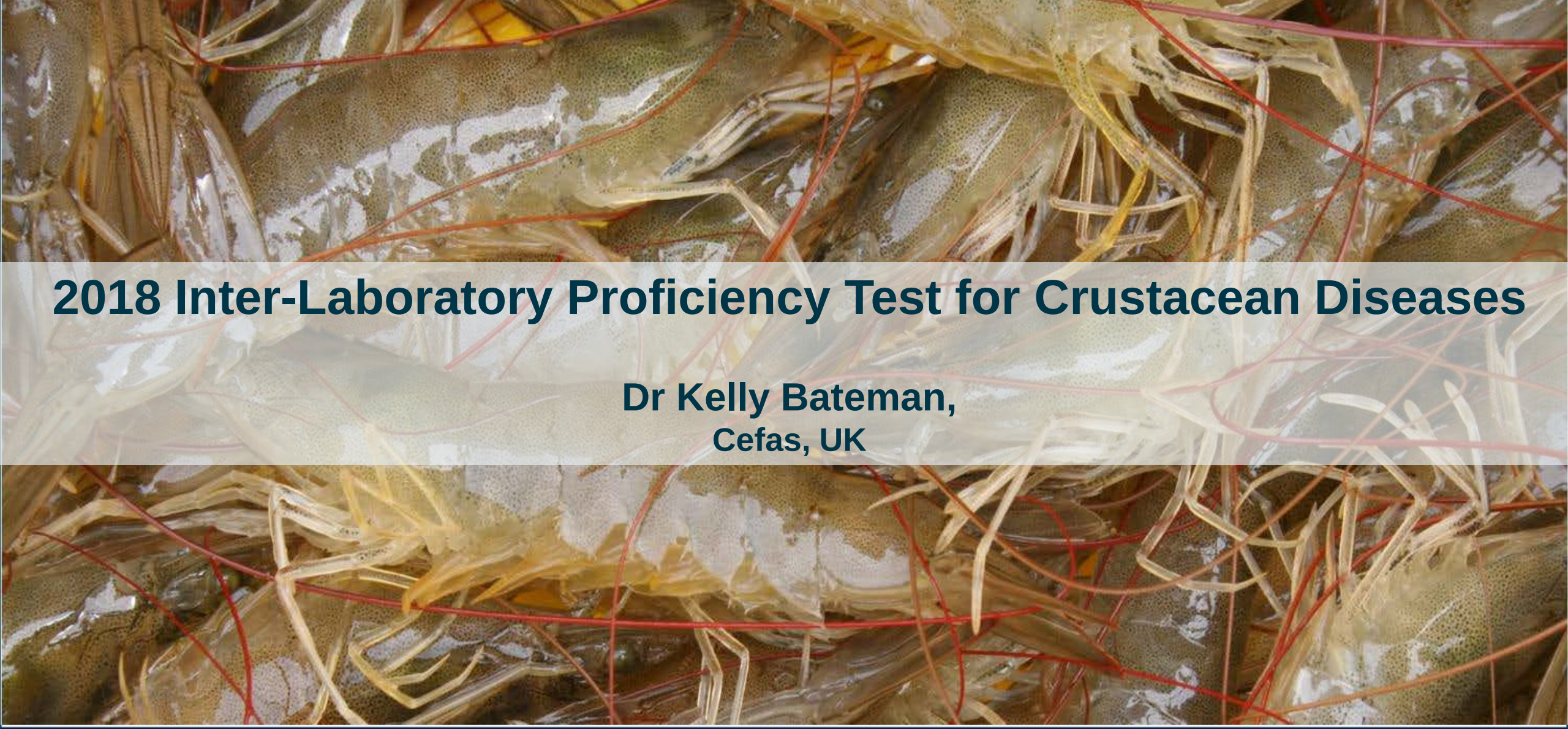

2018 Inter-Laboratory Proficiency Test for Crustacean Diseases

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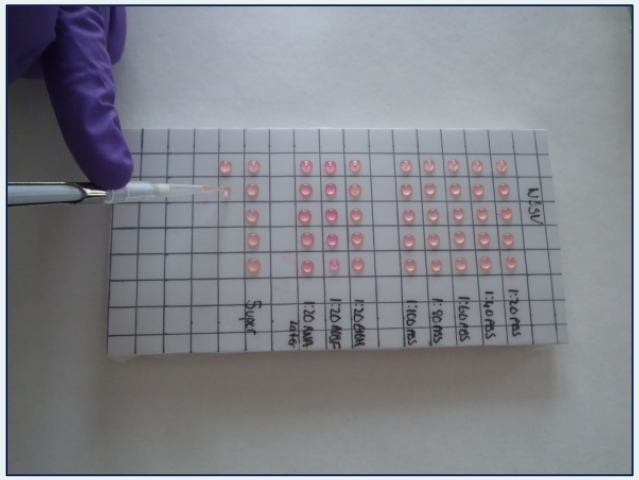
Sample Preparation - Pleopods

- SPF *P. vannamei*
- Pleopods sampled in ethanol
- PCR negative for WSSV, TSV and YHV

- WSSV, TSV and YHV passage with SPF *P. vannamei*
- Up to 100% mortality within 2 days – WSSV
- Up to 100% mortality within 5 days – YHV
- Pleopods sampled in ethanol for WSSV
- Pleopods sampled in RNA Later for TSV and YHV

Sample analysis and distribution

- 5 sets pleopods per shrimp
- 1 set analysed by EURL
- Multiple NRLs received pleopods from same shrimp



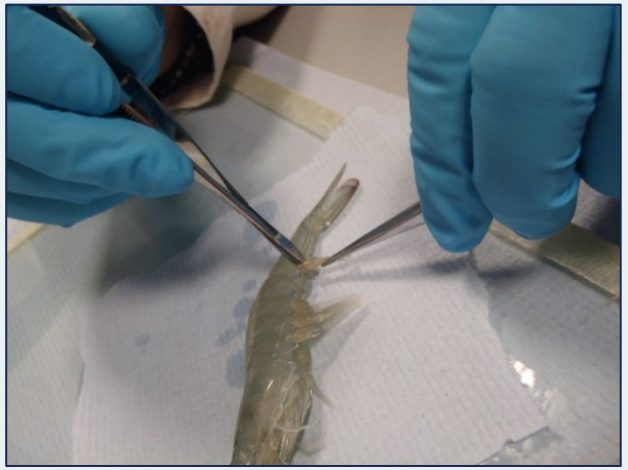
Sample Preparation - Lenticules

- SPF and WSSV shrimp homogenised
- SPF and WSSV supernatant dilutions mixed with lenticulating fluid
- Aliquoted onto parafilm in 25µl volumes
- Refrigerated for 1 week, then transferred to -20°C
- Lenticules are placed in vials containing silica crystals
- Enables the suspension to be transported at ambient temperature

Sample analysis and distribution

- 10% of each sample set analysed by EURL
- Multiple NRLs received lenticules generated from same batch of material

Proficiency Test 2018



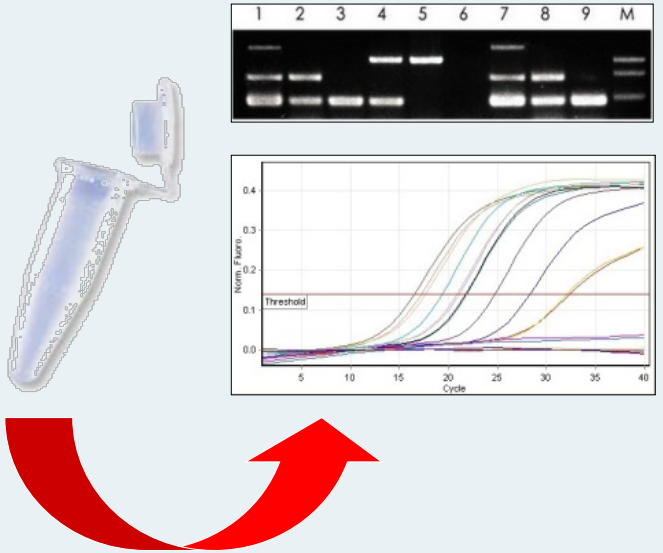
- Multiple NRLs received pleopods from same shrimp
  - 2 pleopods per tube
  - 5 tubes per shrimp
  - 1 tube sampled by Cefas
  - 4 tubes sent to 4 different laboratories
- All NRLs received lenticules from the same batch
  - 10% of batch tested at Cefas
- All tests were shipped via courier and were delivered within 3 days

Participation and Distribution

- Invites sent to 26 National Reference Laboratories (NRLs) in 24 Member States

WSSV
- Samples were sent to 24 NRLs in 22 Member States
- 2 NRLs declined to participate

TSV/YHV
- Samples were sent to 14 NRLs in 13 Member States
- 12 NRLs declined to participate



WSSV Expected Results

Sample ID	Sample Type	Nested PCR Results[1]	qPCR Results[2]
RA18005 - 1	Shrimp Pleopods	Positive	Positive Average CT 15
RA18005 - 2	Shrimp Pleopods	Negative	Negative -
RA18005 - 3	Shrimp Pleopods	Positive	Positive Average CT 15
RA18005 - 4	Lenticule disc	Negative	Negative CT 26
RA18005 - 5	Lenticule disc	Negative	Negative -
RA18005 - 6	Lenticule disc	Positive	Positive CT 26

WSSV Proficiency Test Results Summary

- 24 labs participated from 22 Member States
- Results received from all participating NRLs
- 20 labs 100% correct
- 2 labs correctly diagnosed 5/6 samples, fresh samples sent, 100% correct
- 2 labs correctly diagnosed 4/6 samples, fresh samples sent, 100% correct

- 12 laboratories used Nested PCR methods
- 11 laboratories used real time PCR
- 1 laboratory used multiple methods

TSV/YHV Expected Results

Sample ID	Sample Type	TSV Results[1]	YHV Results[2]
RA18006 - 1	Shrimp Pleopods	TSV Positive	Negative
RA18006 - 2	Shrimp Pleopods	Negative	Negative
RA18006 - 3	Shrimp Pleopods	Negative	Negative
RA18006 - 4	Shrimp Pleopods	Negative	YHV Positive
RA18006 - 5	Lenticule disc	Negative	Negative
RA18006 - 6	Lenticule disc	TSV Positive	Negative
RA18006 – 7	Lenticule disc	Negative	YHV Positive
RA18006 – 8	Lenticule disc	Negative	Negative

TSV/YHV Proficiency Test Results Summary

- 14 labs participated from 13 Member States
- Results received from 13 labs
- 8 labs 100% correct
- 4 lab correctly diagnosed 7/8 samples, fresh samples sent to 3 NRLs
  - 1 lab reported samples in wrong order
  - 2 labs did not complete re-test within the period of the proficiency test
  - 1 lab correctly diagnosed all samples
- 1 labs correctly diagnosed 6/8 samples, fresh samples sent, 100% correct
  - Lab reported problems with contamination and non-specific amplification with the initial YHV testing
- 1 lab did not test the samples which were received

Misdiagnosis Investigation

Sample set	Tube 1	Tube 2	Tube 3	Laboratory Code	Result
2	EURL18002 – 1c	EURL18001 – 1c	EURL18002 – 8b	16	100%
3	EURL18002 – 2b	EURL18001 – 1d	EURL18002 – 8c	23	83%
4	EURL18002 – 2c	EURL18001 – 1e	EURL18002 – 8d	5	100%
5	EURL18002 – 2d	EURL18001 – 2b	EURL18002 – 8e	20	100%
6	EURL18002 – 2e	EURL18001 – 2c	EURL18002 – 9b	8	100%
7	EURL18002 – 3b	EURL18001 – 2d	EURL18002 – 9c	18	100%
8	EURL18002 - 3c	EURL18001 – 2e	EURL18002 – 9d	15	83%
9	EURL18002 – 3d	EURL18001 – 3b	EURL18002 – 9e	19	100%

- 5 pleopod tubes per shrimp
- 1 pleopod set analysed by Cefas
- Multiple NRLs received pleopods from same shrimp

- Red text – incorrect diagnosis
- Green text – correct diagnosis, multiple labs

- No consistency between incorrect diagnosis

What Happened?

- Contamination issues?
- Which step does contamination occur?
- Discussion at NRL meeting

Contact

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http://www.cefas.co.uk