Accreditation of White Spot Syndrome Virus (WSSV)

23rd Annual Workshop
27-29th of May

Argelia Cuenca — Molecular Biologist
Camilla Priess — Research Assistant

Background

- Workplan: get accreditation for:
  - Taura Syndrome virus
  - Yellow-head virus

  } RNA

  - **White spot syndrome virus** - DNA

Work in progress!

Workflow, results, next steps… suggestions?

Diagnostic PCR assay

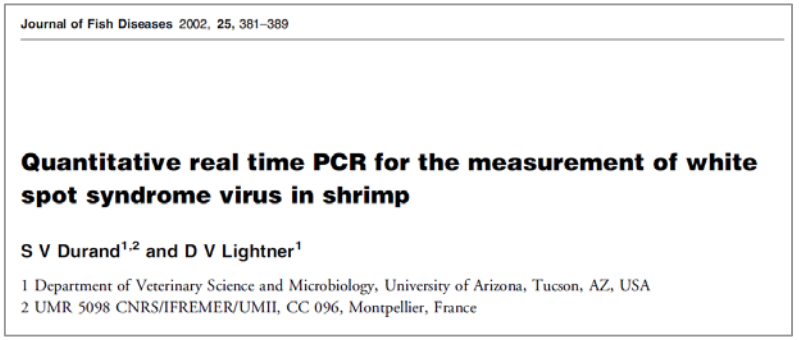
OIE:

- Nested PCR (Lo et al., 1996)
- TaqMan-based qPCR (Nunan & Lightner, 2002)

Vol. 25: 133–141, 1996 | DISEASES OF AQUATIC ORGANISMS Dis Aquat Org | Published May 9

Detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction

Chu-Fang Lo[1,*], Jiann-Horng Leu[2], Ching-Hui Ho[1], Chau-Huei Chen[1], Shao-En Peng[1],



Journal of Fish Diseases 2002, 25, 381–389

Quantitative real time PCR for the measurement of white spot syndrome virus in shrimp

S V Durand[1,2] and D V Lightner[1]

1 Department of Veterinary Science and Microbiology, University of Arizona, Tucson, AZ, USA
2 UMR 5098 CNRS/IFREMER/UMII, CC 096, Montpellier, France

EU:

- Modified nested PCR + sequencing
- "Alternative approaches … which offer equivalent sensitivity and specificity to those described in this part may be applied."

Diagnostic PCR assay

OIE:

- Nested PCR (Lo et al., 1996)
  - Risk of false positive
  - Long run time (2×168 min)

	Cycles	Temp.	Time
Initial Denaturation		94°C	3 min
Denaturing	40	94°C	1 min
Annealing		55°C	1 min
extension		72°C	2 min
Final extension		72°C	5 min

EU:

- Modified nested PCR
  - Increased specificity
  - Fast run time (2×49 min)

	Cycles	Temp.	Time
Initial Denaturation		94°C	2 min
Denaturing	30	94°C	30 sec
Annealing		62°C	30 sec
extension		72°C	30 sec
Final extension		72°C	2 min

Diagnostic PCR assay

- Nested PCR or qPCR…

	Nested PCR	qPCR
Sensitivity	20 copies	4 copies
Time	5-6 hours	3-4 hours
Steps	preparation + 2×run + gel	preparation + run
	Higher contamination risk	

Analytical validation

Validation steps

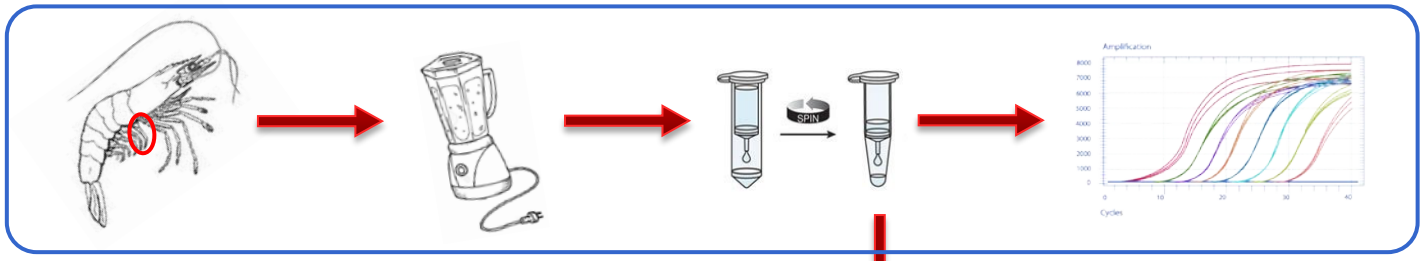
- Efficiency & detection limit (sensitivity)
- Analytical specificity
- Robustness
- Reproducibility
- Repeatability

Analytical validation

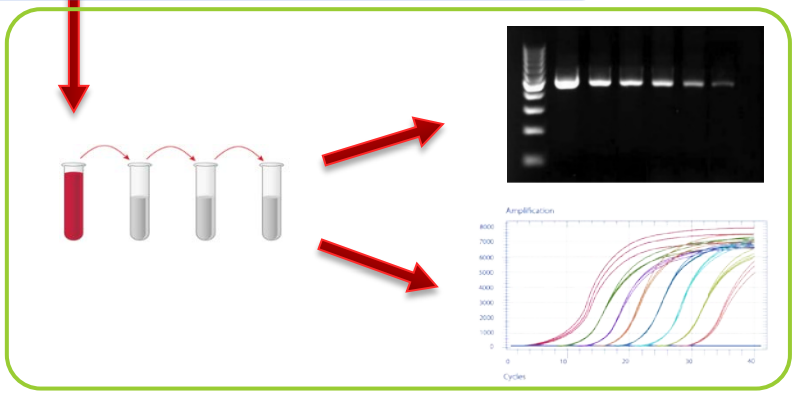
Detection limit of WSSV

- Positive control: WSSV DNA fragment (GenBank U50923) → gBLOCK
- 10-fold dilutions of gBLOCK → test both methods (10^{-10})

- Proficiency test → WSSV-injected shrimp with low Ct-value
- 10-fold dilutions (samples + gBLOCK) → test both methods



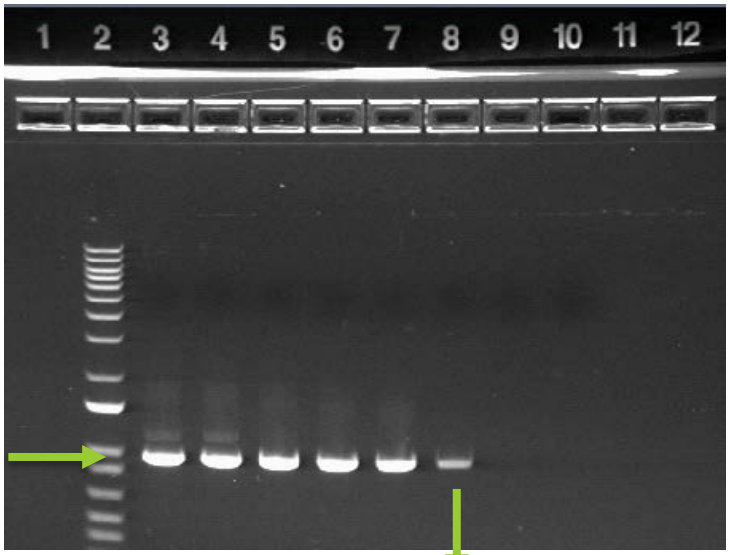
Proficiency test



Analytical validation

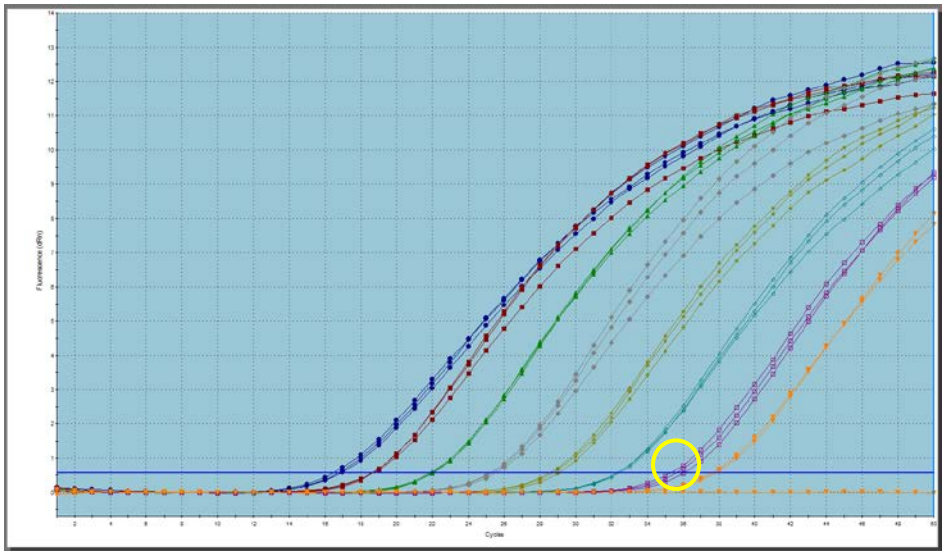
Detection limit of WSSV

Nested PCR



WSSV 10^{-5}
≈ 270 copies

qPCR



Efficiency = 106.4 %

WSSV 10^{-6}
≈ 27 copies

Analytical validation

Validation steps

- Efficiency & detection limit (sensitivity)
- Analytical specificity
  - Crustacean DNA virus
  - Taura Syndrome virus
  - Yellowhead virus
- Robustness
- Reproducibility
- Repeatability

Analytical validation

Validation steps

- Efficiency & detection limit (sensitivity)
- Analytical specificity
- Robustness
- Reproducibility
- Repeatability

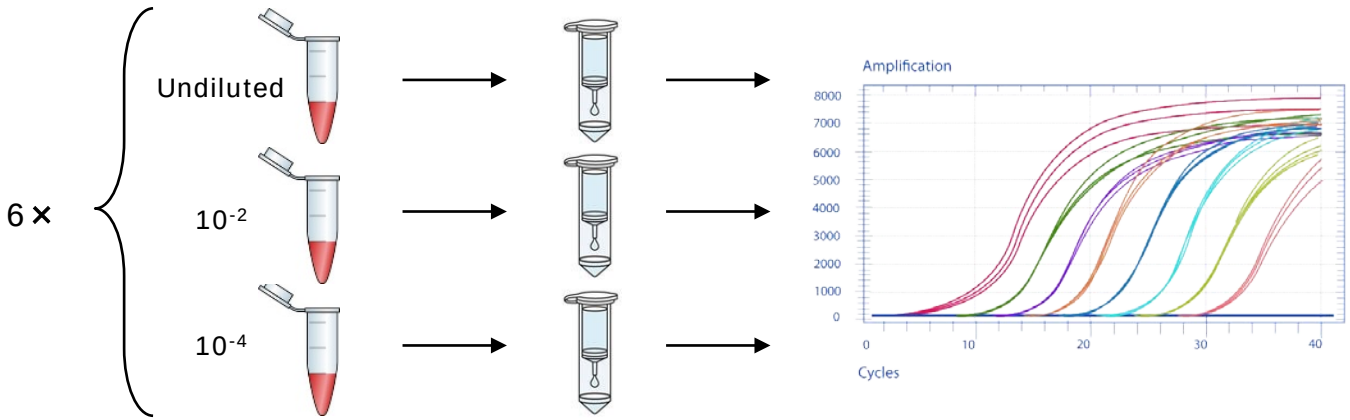
	-25% master mix	Recommended	+25% master mix
-25% primer/probe	18.55	18.29	18.22
Recommended	18.44	18.23	18.24
+25% primer/probe	18.28	18.41	18.50

- Samples in triplicates
- Mean Ct-value = 18.35

Analytical validation

Validation steps

- Efficiency & detection limit (sensitivity)
- Analytical specificity
- Robustness
- Reproducibility
- Repeatability



Analytical validation

Validation steps

- Efficiency & detection limit (sensitivity) ✓
- Analytical specificity – to do!
- Robustness ✓
- Reproducibility – working on it!
- Repeatability - to do!