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The use of field diagnostic qPCR in gill health monitoring.

Alan Dykes

Galway, 16th April, 2015

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Site Staff

- First defence - eyes & ears
- Appetite
- Behaviour
- Gill scores
- Environmental monitoring

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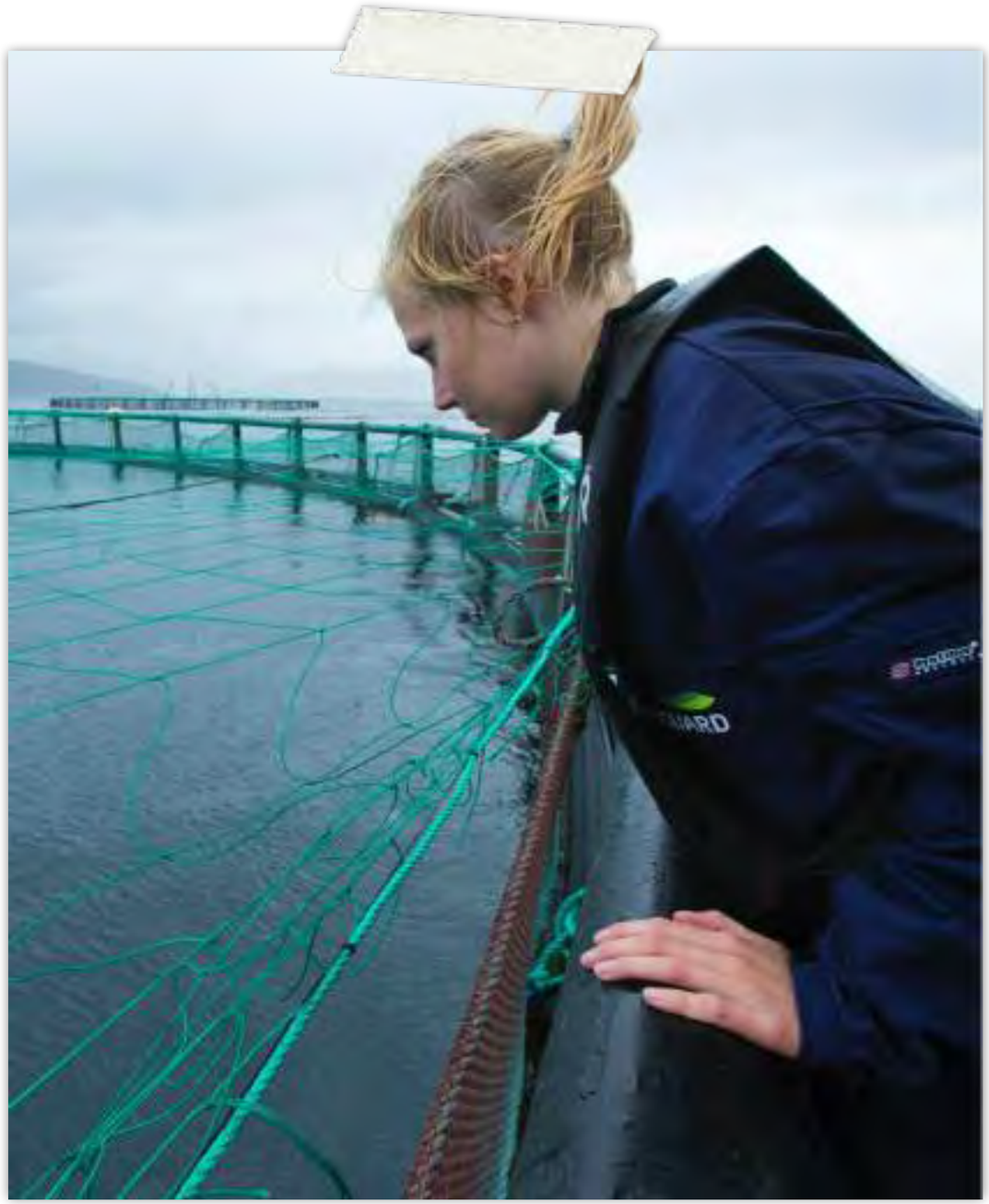
Fish health personnel

- In-house or 3rd party
- More specialised
- More experienced (normally) in fish health matters
- On site diagnostics..



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Light Microscope

- Relatively cheap USB enabled microscopes either on site or brought on site by 3rd party

- Useful for confirming presence/absence of amoeba

- Useful for confirming PGD

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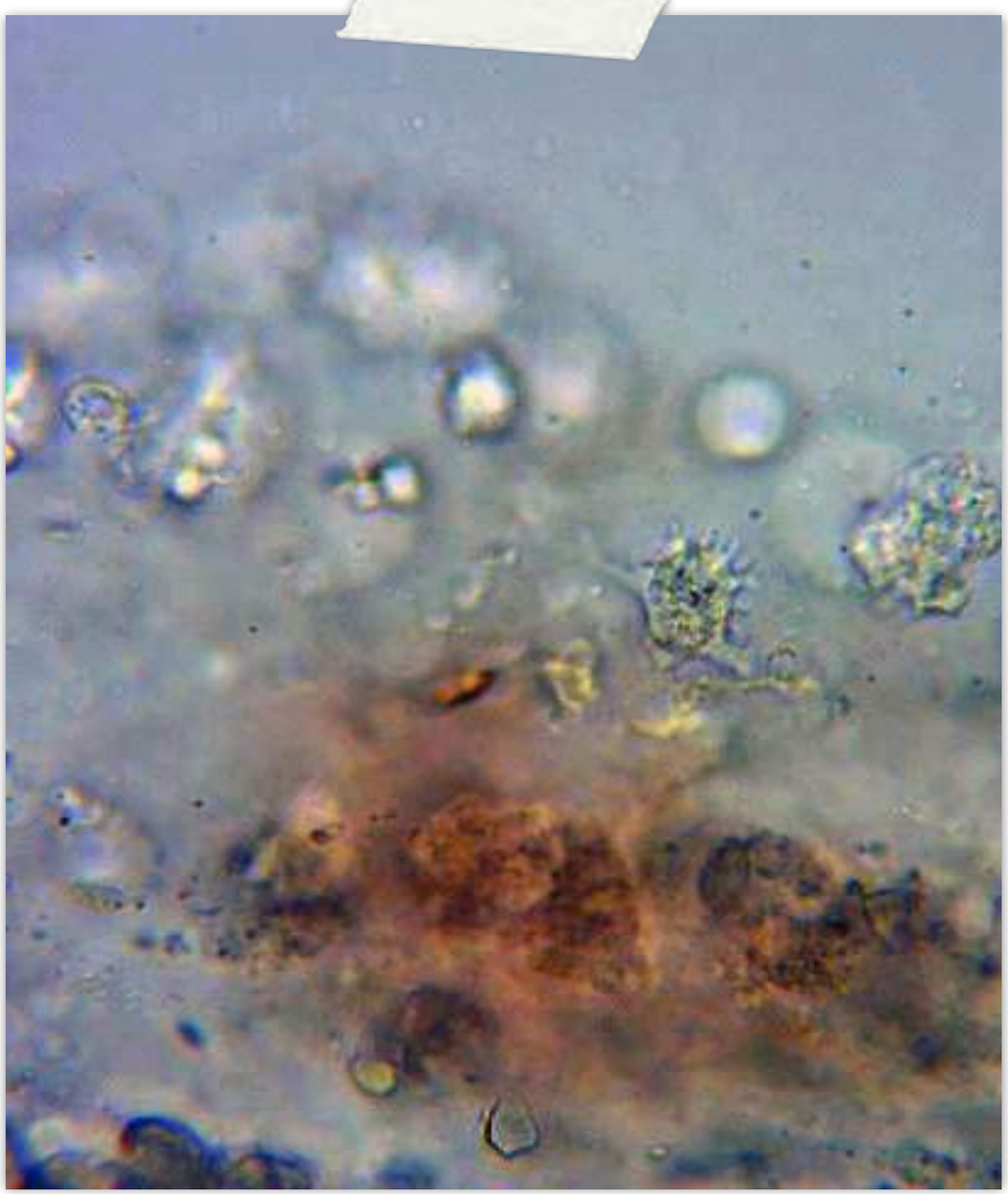
AGD



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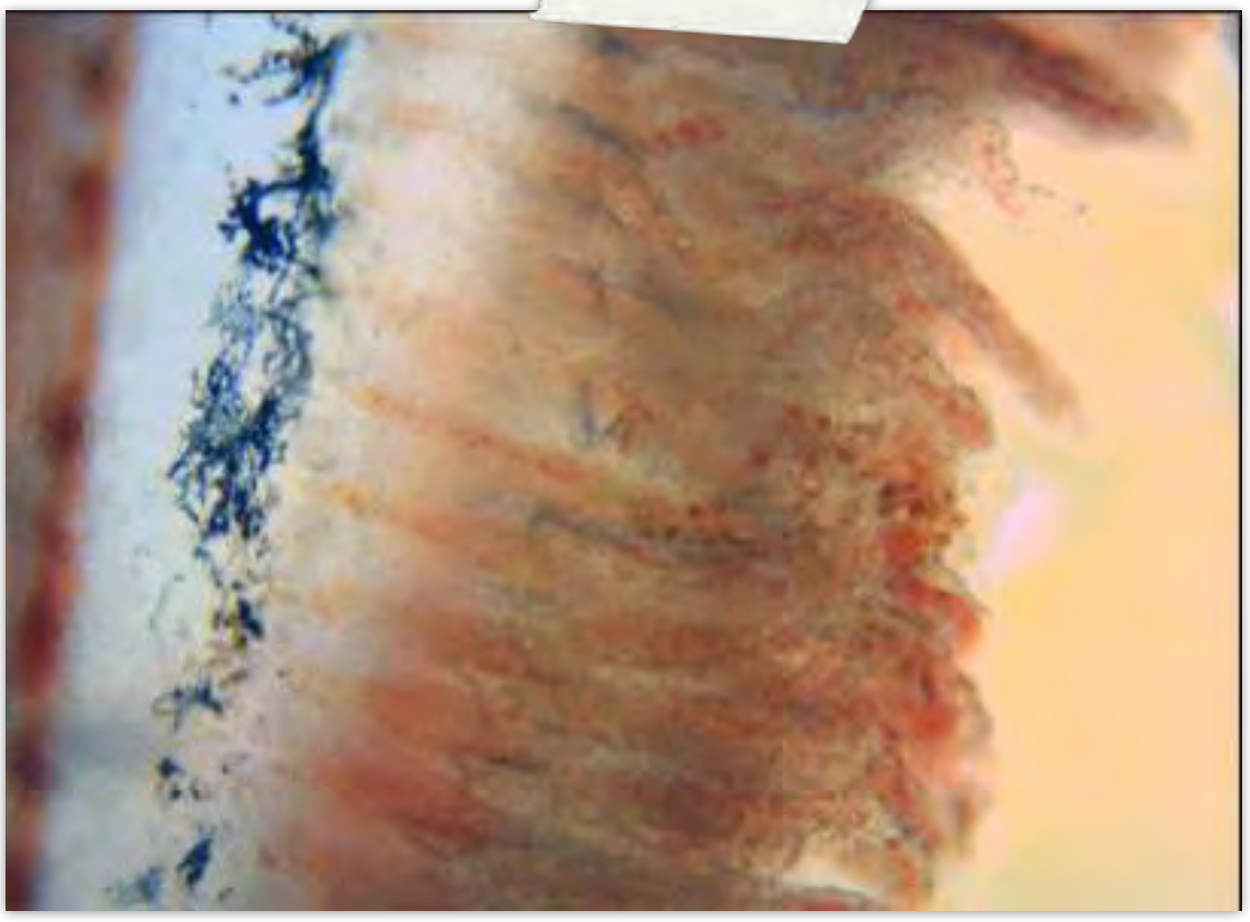
PGD/PGI



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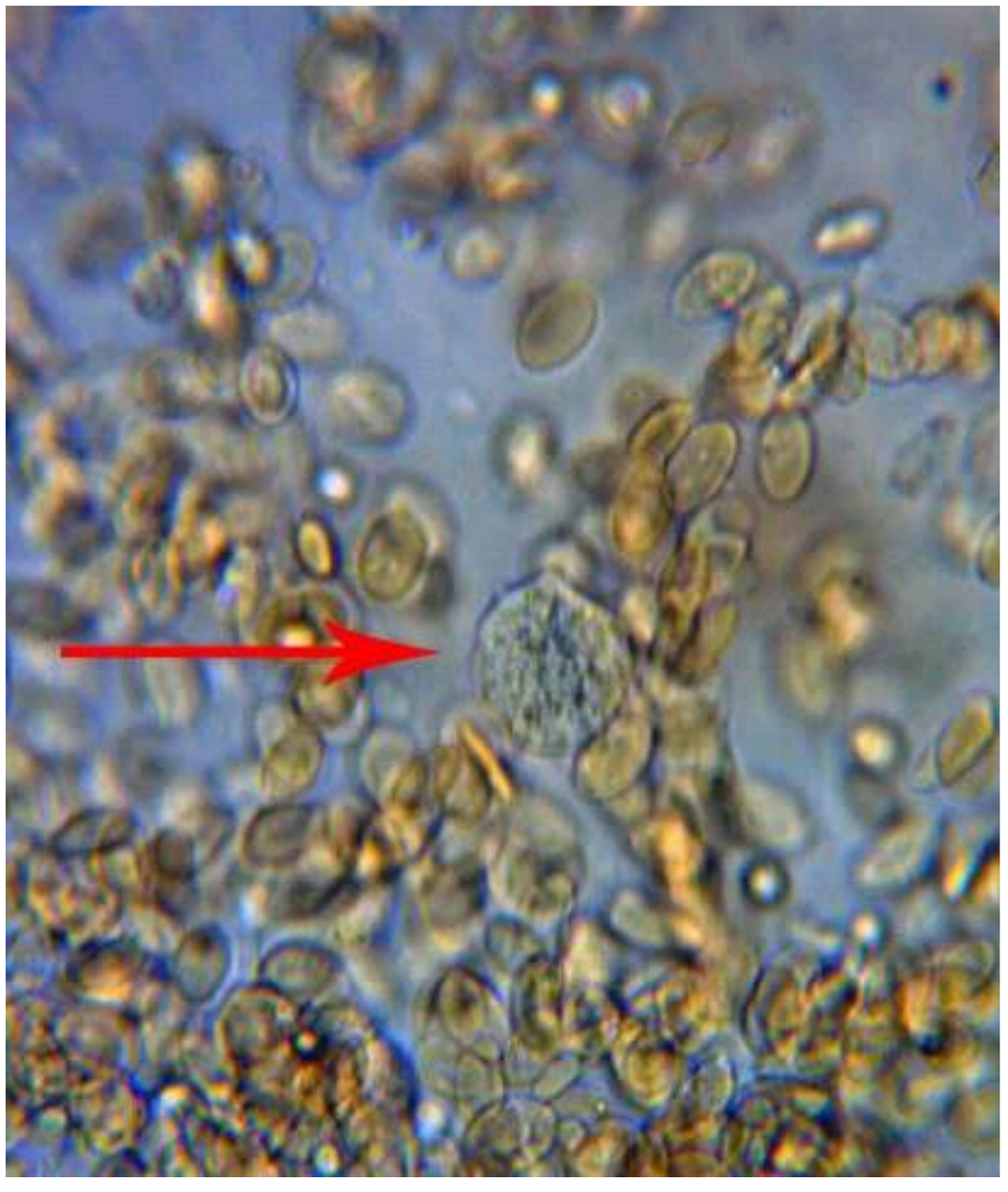
Multifactorial

- Ca. Branchiomonas cysticola
- Ca. Branchiomonas salmonis
- Desmozoon lepethophterii
- Paramoeba perurans
- Parvicapsula pseudobranchicola



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Sample……and wait…….

- No reliable on-site field detection platform

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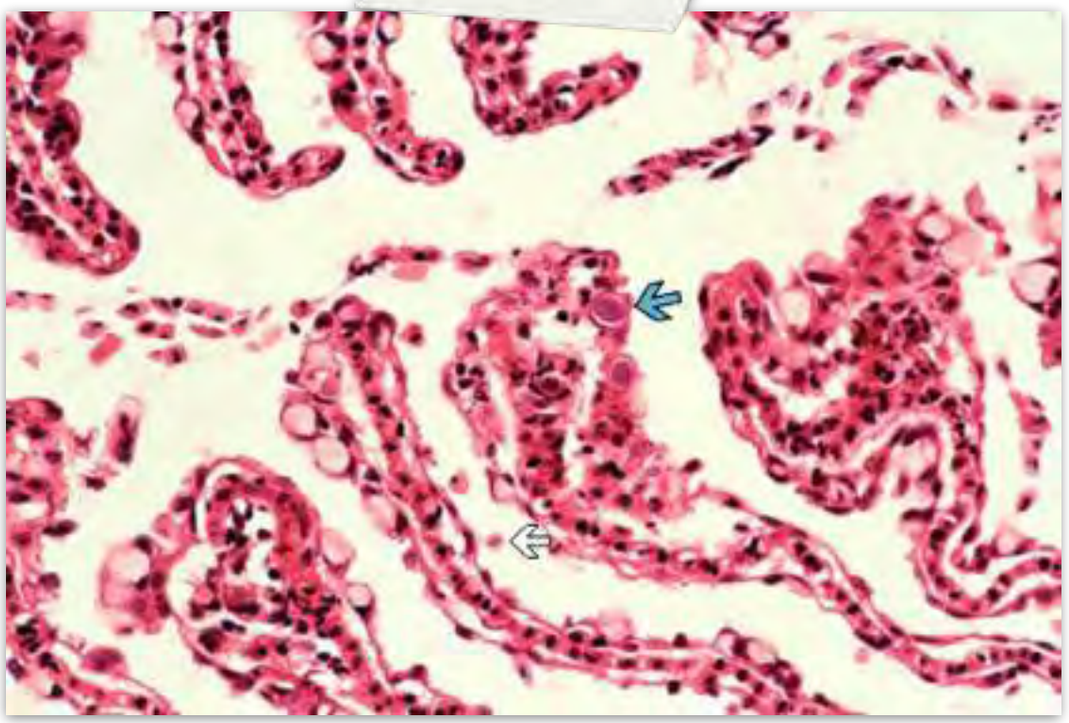
Interpretation

- Sample results may be from different laboratories
- Histology again, from another source
- Diffusion of diagnostic focus
- Can lead to requests/advice for further sampling



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A gap identified and a fortuitous meeting

- Late 2013 a fortuitous meeting at Stirling
- TSB funding to develop a point of need diagnostic platform for viral diseases

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Accredited machine (under development)

Point of need diagnostics for viral disease in salmon aquaculture

Michael McGowan[1], Simon MacKenzie[1], Manfred Weidmann[1] and Nikos Steiropoulos[2] and Alan Dykes[3].

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2. Europharma Scotland Ltd.
3. Fishguard UK

UNIVERSITY OF STIRLING

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Europharma

Introduction

Salmon alphavirus (SAV), Piscine reovirus (PRV) and Piscine myocarditis virus (PMCV) have caused major detrimental impacts on the salmon aquaculture industry throughout Europe, limiting growth and profit due to high mortality of fish. Each virus induces almost identical symptoms and morphological impacts in salmon, rendering distinction through current diagnostic methods, extremely slow, difficult and inaccurate. Currently no rapid detection platforms exist to quickly identify and distinguish between each virus. The reliable detection and identification of smoltification in salmon is another major issue preventing growth for the industry due to mortality of fish from early sea transfer, and de-smoltification of fish due to late transfer. mRNA transcripts associated with smoltification have been explored but currently no detection platform to monitor smoltification is available. This project aims to develop a mobile qRT-PCR platform (Smart Cycler) to be used at fish farms as a point of need detection system. We will incorporate a Taqman multiplex assay for detection of all three viruses and smoltification biomarker, GAPDH and NaKATPase, assays to the Smart Cycler to enable on site detection and measurement.

SUMMARY

- **Two specific and sensitive assays for detection of GAPDH and NaKATPase were developed and transferred into a mobile Smart Cycler detection device for onsite detection.**
- **Three specific and sensitive individual assays for detection of SAV, PRV and PMCV were developed for future integration into a multiplex assay for use in a mobile Smart Cycler detection device for onsite detection.**

Methods

1) Development of quantitative RNA Standards
For each assay target genes were cloned into plasmids and then transcribed using promoters (SP6/T7) to generate quantitative standards to test the analytical sensitivity and specificity of the assays.

2) Smoltification marker assays
The design of the qRT-PCR was optimised using existing targets for both genes. The analytical sensitivity and specificity of quantified standards was then tested using a Mx3005P qPCR system. The assay was transferred to a mobile Smart Cycler system (figure 1), adapted to a fast protocol and the analytical sensitivity was then verified. Assays will now be evaluated using archived mRNAs from fish sampled throughout smoltification and then validated on farm.

3) SAV, PRV, PMCV
Primer design for the target regions of each virus were generated by aligning all available sequences from Genebank in a clustal V alignment. The analytical sensitivities of the individual assays were tested as described above. A multiplex assay incorporating all three viruses for detection assays will be optimised in the lab and transferred onto the mobile system. The individual and the multiplex assays will be tested on archived fish material first and then on farm.

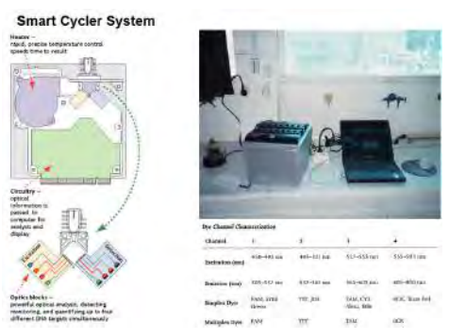

Figure 1. An internal diagram demonstrating how of the Smart Cycler system operates (left). A fully set up smart cycler system ready for use (top right). Table indicating Dye channel characterisation (bottom right)

Results

- All target amplicons were successfully cloned, transcribed, and quantified RNA standards generated for all targets.
- All virus assays for the detection of SAV, PRV and PMCV standards showed an analytical sensitivity of 10 RNA molecules detected (figure 2 A,B and C)
- GAPDH and ATPase standards were successfully transferred to the SC giving an analytical sensitivity of 100-1000 RNA molecules detected (Figure 2 D and E).

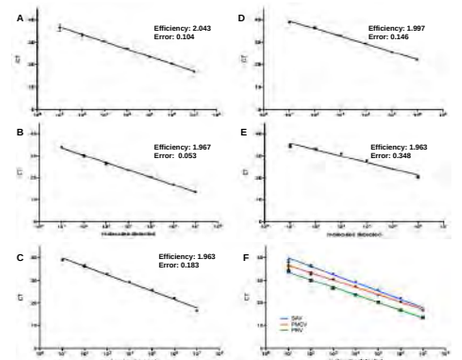

Figure 2: Standard curves for (A) PMCV (104.3%, SE 0.104), (B) PRV (96.7%, SE 0.053), (C) SAV (96.3%, SE 0.183), (D) ATPase (99.7%, SE 0.146) and (E) GAPDH (96.3%, SE 0.348). Graph (F) Compares the three viral standard curves (SAV, PRV and PMCV)

Viral/gene target	Sensitivity * Real-time-PCR 3 Runs	Sensitivity * Smart Cycler MX 3 Runs	Sensitivity Smart Cycler SC 3 Runs
SAV	10^1	N.D	N.D
PMCV	10^1	N.D	N.D
PRV	10^1	N.D	N.D
ATPase	10^2	10^2	10^2
GAPDH	10^2	10^2	10^2-10^3

Table 1: Sensitivity of rtQPCR assays .* molecules detected. MX; slow protocol, SC;fast protocol

Conclusions

- Highly efficient qRT-PCR assays for the detection of SAV, PRV and PMCV were developed
- Efficient qRT-PCR assays for detection of GAPDH and ATPase were successfully developed and transferred onto the Smart Cycler.
- Fast protocols (30min) are as efficient as traditional QPCR protocols and will provide rapid on site turnaround times.

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Results so far

3 target viral fish disease agents successfully ID'd in samples. Machine used in Africa for field testing Ebola.

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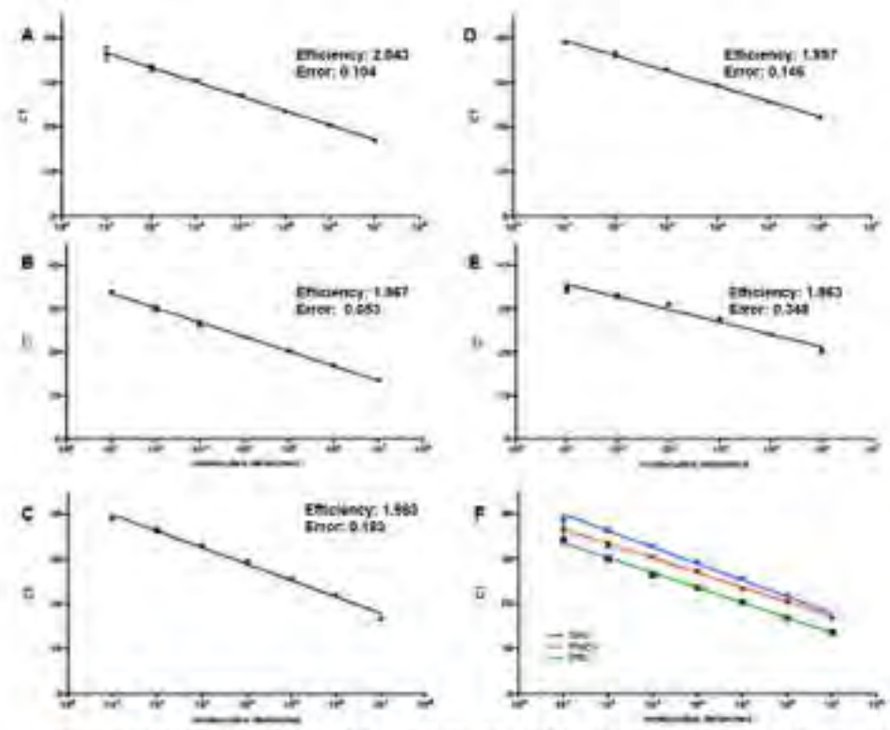

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Portable to a degree

Everything fits in the boot of a family car. New technology being developed at the moment means the equipment will shrink in size and also be faster to operate.

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A gap identified and a fortuitous meeting

- Late 2013 a fortuitous meeting at Stirling
- TSB funding to develop a point of need diagnostic platform for viral diseases
- Company called Primerdesign Ltd gets in touch to demonstrate the Genesig q16, an ultraportable, 16 well, quantative real time thermocycler.
- First primers developed late 2014
- Field trials currently underway

genesig® q16

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Reliable Rapid Diagnostics. Everywhere.

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brings qPCR diagnostics to the farm

Fishguard Fish Health Services brings to the Scottish Aquaculture Industry a revolutionary step forward in on-site detection of viral, bacterial and parasitic pathogens. Our ultraportable qPCR platform, genesig® q16:

- Delivers reliable point-of-need diagnostics, improving the dialogue between the fish health professional and the farmer
- Allows for prompt discrimination of pathologies with similar symptoms
- Speeds up the decision-making process with results in just 2 hours

For more information please contact the Fishguard team at 0141 4357100 or mail@fishguard-uk.com

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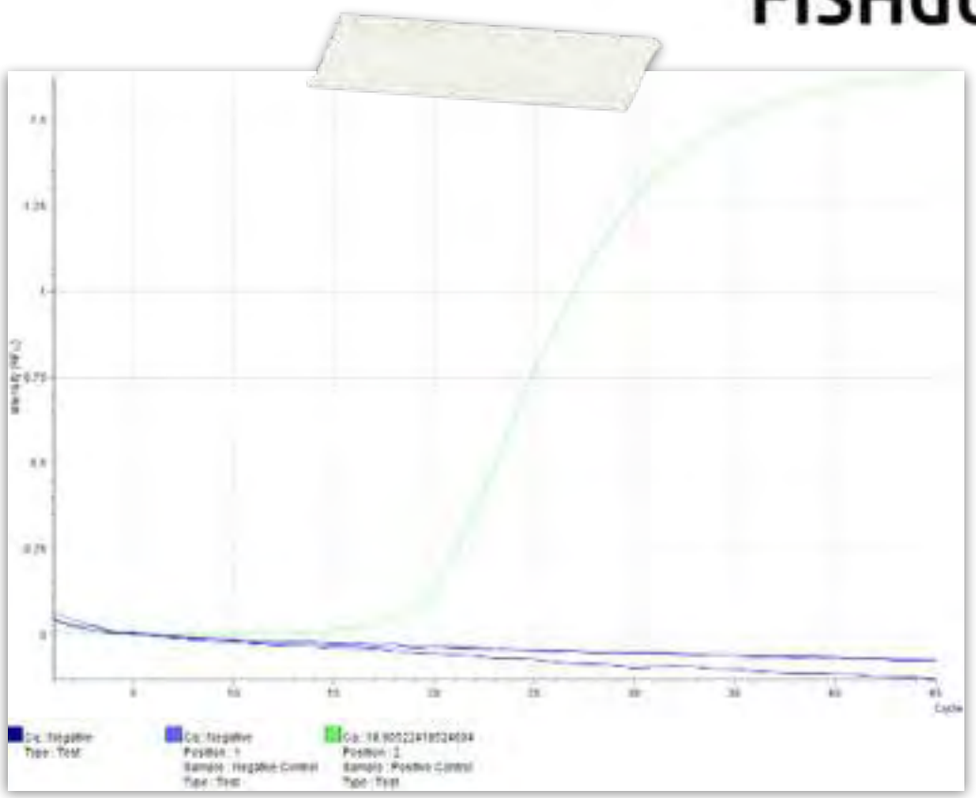
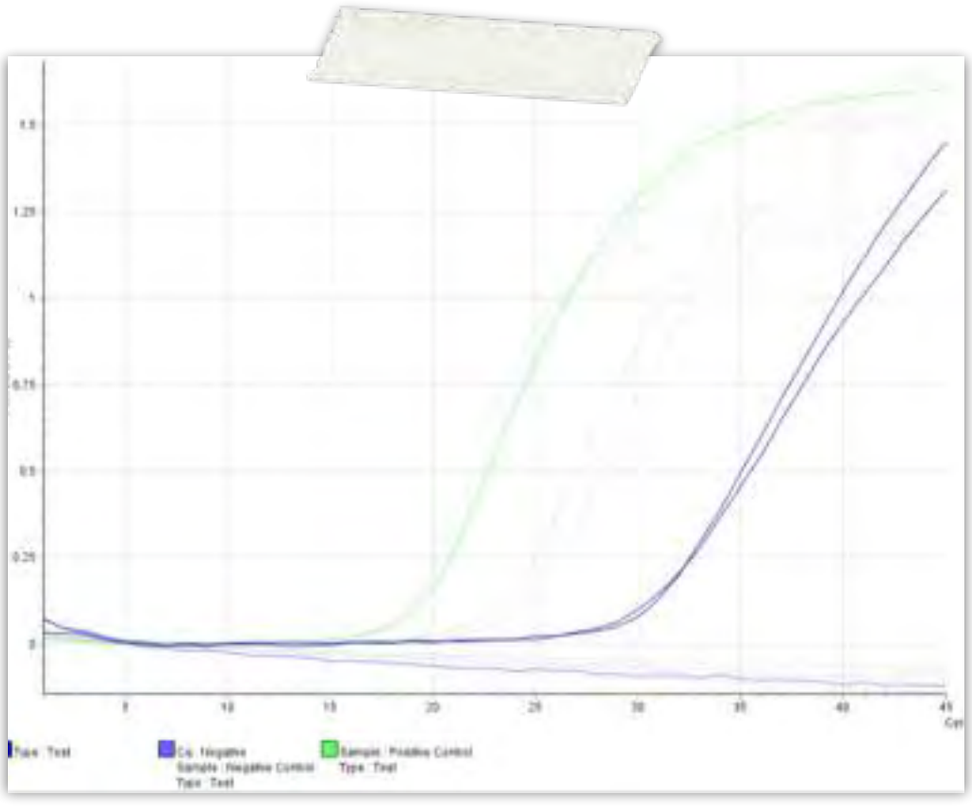
genesig® q16

Last weeks trials using samples from perurans positive and negative sites. So far so good. All field testing has been benchmarked against existing labs.





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Instant dialogue

- On site result to confirm/refute suspicion
- Valuable dialogue between fish health professional and site staff
- Ability to take rapid decisions regarding starvation/treatment/ mitigation
- Worst case scenario - instant flagging for investors
- Knowledge harvesting

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The future..every shore base..every sea site…….