IHNV QPCR Validation at MSS

Accreditation

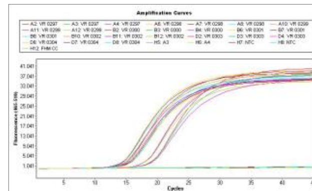
- MSS Diagnostic Group operates under accreditation by the United Kingdom Accreditation Service (UKAS) under the ISO 17025 standard.
  - All measurements traceable to national or international standards
- Molecular genetics department also has UKAS Flexible Scope accreditation
  - Allows new or modified methods to be added to the schedule of accreditation quickly following a robust validation procedure
  - Requires more active involvement by quality and technical management

Standard method
Molecular detection of RNA pathogens

- RNA extraction: Qiagen RNeasy Plus automated using Qiacube
  - Spin column method
  - 10 mg tissue extracted
- RT-QPCR: Qiagen One step RT-PCR real time mix using the Roche Lightcycler 480 platform, run for 45 cycles
  - Endogenous control ran alongside pathogen assay
  - One step method accredited in 2013
  - One step method increased sensitivity overall
  - All current pathogen and endogenous control assays assessed during one step validation including ISA, SAV and VHSV
- New assays can be added under flexible scope following validation
  - Validation procedure based on MIQE guidelines

Initial Feasibility Study

- Suite of IHNV isolates tested in triplicate
  - Strains FR32/87, IT-217A and the G24 isolates



- Covered all genotypes, all successfully detected
- Only cell culture material available, ideally would like to test assay with tissue matrix to ensure no inhibition

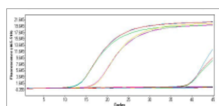
Specificity

Range of other RNA viruses tested including

- Other fish rhabdoviruses: VHSV (Genotypes Ia, Ib, II, III and IV), Spring Viraemia of Carp Virus, Perch Rhabdovirus, Tench Rhabdovirus, Grass Carp Rhabdovirus, Snakehead Rhabdovirus, Hirame Rhabdovirus
- Listed RNA viruses: ISAV, Nodavirus
- Commonly isolated RNA viruses: SAV, IPNV

Perch Rhabdovirus DK-6389 gave Cp values of >40 in all three replicates

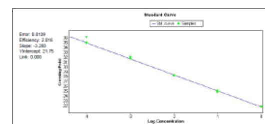
- Suspect contaminated sample
- Currently regrowing isolate
- Only have a single isolate in our culture collection
- Not tested in Purcell paper



Reaction Efficiency & Analytical Sensitivity

Serial dilution run in triplicate for each genotype

- Efficiency curves generated
  - 90 to 105 % efficiency required for assay to be deemed suitable
  - All within range
  - Only cell culture matrix used as no tissue samples available



- Limit of Detection / Assay range calculated
  - Average Cp value obtained from the highest dilution generating a positive result in all replicates is defined as the limit of detection
  - Variation in assay range is always observed between samples
  - In practice, test is performed in triplicate for 45 cycles and all three replicates must produce a Cp value for a sample to be reported as positive

Repeatability

- Assay repeatability
  - A minimum of 5 replicate samples are prepared
  - Tested by three operators, of varying experience, on three separate days.
  - Must be within three cycles
- External assessment of the assay
  - Covered by EU ring test
  - IHNV dedicated ring test ideal
    - Varying concentrations / pathogen load

Positive Control Development

- All accredited QPCR pathogen assays have an artificial positive control to assess assay performance
- Contains pathogen assay primer and probe (FAM) sequences plus a unique probe (VIC) sequence

FORWARD PRIMER	PATHOGEN PROBE	ARTIFICIAL PROBE	REVERSE PRIMER

- Multiplex assay allows differentiation between true positive and positive control contamination
- Currently validating movement of positive control development from in house to commercial company

Development of a widely applicable positive control strategy to support detection of infectious salmon anaemia virus (ISAV) using Taqman real-time PCR

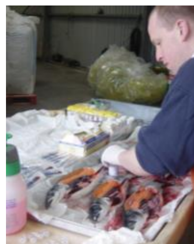
M Snow, P McKay and I Matejusova

FRS Marine Laboratory, Aberdeen, UK

Further Validation ?

Field validation comparing virus isolation, histopathology and RT-QPCR tests?

- E.g. BKD, ISA and SAV
- Need access to samples
- Expensive



Effect of pooling samples?