Summary of Information from a Document Review and On-Site Visit (November 18, 2011) for the
ISA OIE Reference Laboratory at Atlantic Veterinary College

Procedure or Element Reviewed	Details	Comments
Quality management or Quality assurance procedures	The lab provided some key SOPs related to the method and procedures used for analysis of the samples, however some that would be expected in a lab doing diagnostic testing were not provided. These include; calibration and maintenance of key equipments such as pipettors, suitability and tracebility of reagents and materials used in analysis, result reporting.	The lab has started to develop a quality management system, but many elements are not yet in place.
Cleaning and decontamination	Cleaning and decontamination before and after working on samples was reported as always being done, and is specified in SOP#ISAV-R RT-PCR-12-08 Infectious Salmon Anemia Virus Real Time RT-PCR (TagMan Assay). No requirement for environmental monitoring was included.	There were no records of cleaning, decontamination or environmental monitoring.
Documents	SOPs were provided for the real time RT-PCR ISAV method and related processes (RNA extraction setting up a standard curve). SOPs for Cell culture were also provided. No details for reporting results are included in the SOPS	For diagnostic testing, instructions for result reporting should be included in an SOP either with the method or in a separate document.
Records	Records related to these sample were in an Excel sheet but did not include all the steps nor the equipment used . Only one analyst was involved.	Lack of records for all steps and equipment used make it difficult to identify where an error or problem might have occurred, and where remedial or corrective action might be required.
Training	No procedure for method or protocol specific training was provided	Records that were provided showed more general qualifications and expertise.There were no records (such as results from blind panels) demonstrating analyst competence in the specific method used for the samples in question.

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Sample receiving Room 329S	The room where the fish samples were initially received is also used for inoculating fish cell lines with ISAV. PCR associated with cloning and sequencing of ISAV has also been carried out in this room.	A small potential for cross contamination exists.
RNA extraction Room 326S	Combination of Trizol + RNeasy Mini Kit - whole tissue and homogenates. The room and biosafety cabinet where RNA extraction took place (which includes extraction of positive control material) is the same room and biosafety cabinet where RNA template is added to the PCR master mix. Although the biosafety cabinet where both these procedures take place does not have a UV light, the cabinet is disinfected with Virkon between uses. The same set of pipets was used for RNA extraction and addition of template to the PCR master mix.	Potential for cross contamination exists here as well. The biosafety cabinet where extraction of strong positive control material takes place is in the same biosafety cabinet used for adding template to PCR master mix. The same pipets are used for both procedures.Controls and sample are extracted separately - how can one confirm extraction worked?
PCR targets	Segment 8 - primers and probe as desribed in 2009 Manual of Diagnostic Tests for Aquatic Animals - 104 bp product; Segment 7 as in Manual described in protocol; Segment 6 to discriminate between NA and European viruses - confidential IP	
One-step or two-step RT-PCR?	One-step RT-PCR; Tth DNA polymerase + Aptamers provides hot start capabilities and increased sensitivity down to 0.1 pg of total RNA using kit's special enhancer	In theory and in most cases a two-step RT-PCR is usually more sensitive than a one-step RT-PCR
PCR chemistry utilized	LC480® Master Hydrolysis Probe (Roche)	
Primer and probe concentrations	Calculations in Master mix table in SOP#ISAV-R RT-PCR-12-08 were confusing and needed clarification (720 nM for primers and 200 nM for probe). Clarification was provided.	Primer and probe concentrations in combination with the chemistries used can affect the analytic sensitivity of PCR assays.

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Internal control	Atlantic salmon elongation factor 1α (ELF1α) is described in the SOP but upon questioning is rarely used. ELF1α internal control was not used in any of these submissions. Neither was RNA quantitation by nanodrop or other spectrophotometric means.	Lack of data makes it difficult to assess the quality of the samples for which positive results were obtained. However, according to Dr. Kibenge, the 48 hearts were received at AVC with the ice packs still frozen suggesting that the specimens were in good condition when they arrived at AVC.
Instrument platform used	Roche LC480	Instrument platforms and associated analysis software can have some affect on analytic sensitivty/specificity and result interpretation
Cycling conditions	63oC x 3 minutes; 95oC x 30 seconds; 45 cycles @ 95oC x 15 sec, 60oC x 1 min, 72oC x 1 sec	The brief RT step in this protocol (3 minutes) may sacrifice assay sensitivity
Interpretation of Ct values	Cts ≤ 30 are considered strong positive; Cts ≥ 30.1 and ≤35 are considered weak positive; Cts ≥ 35.1 and ≤40 are considered very weak positive; Cts ≥ 40.1 and ≤ 45 are considered suspicous; No Ct is interpreted as negative.	The interpretation of these ranges, particularly at the higher Ct values ≥ 35.1 may differ depending on the laboratory. In addition, to the Ct consideration also has to be given to the shape of the curve, Ct of the internal control, etc in the final interpretation.
Validation data?	Reference - Snow et al., 2006. Development, application and validation of a taqman® real-time RT-PCR assay for the detection of infectious salmon anemia virus. In New Diagnostic Technology: Applications in Animal Health and Biologics Controls. Dev. Biol., Basel Karger. 126, 133-145.	
Positive control	ISAV RNA extracted from TO cell culture lysates - Ct values of the positive controls are low (<20) which can be a source of contamination	ISAV RNA is a potential source of cross-contamination. Furthermore, because genomic RNA is used, it makes distinguishing between true positives and contamination with the positive control difficult and dependent on sequencing the RT PCR products..

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Analysts certified to carry out test Proficiency testing	Molly Kibenge is the only analyst that performs diagnostic PCR assays for ISAV. A description of an ISAV RT-PCR Ring Test Phase II with Fish Diagnostic Labs in Chile May 2009 was provided. The values obtained by Molly Kibenge appear to have been used as the expected values.	Did not see proficiency panel results that could demonstare analyst competence.
Cell Culture	Cell cultures were innoculated in 329S. There were no worksheets or logbooks showing the details related to the innoculation.	A lack of records makes it difficult to asess what was done.