Procedure	AVC	DFO	Significance
1. Sample receiving	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 is also carried out in this room.	Separate room used just for sample receiving and initial processing of samples (tissue harvesting, etc). Observed procedures which involved good use of aseptic technique during sample collection.	Small potential for cross contamination?
2. RNA extraction	Combination of Trizol + RNeasy Mini Kit - whole tissue and homogenates. The room and biosafety cabinet where RNA extraction took place w(which includeds extraction of positive control material) as the same room and biosafety cabinet that RNA template was added to the PCR master mix. The same set of pipets was used for both procedures.	TRI Reagent or TRI-LS reagent - whole tissue or 10% tissue homogenates. RNA extraction was run in batches of 30 samples + 2 blanks. Reagents are separated from one batch to another. RNA template is added to PCR master mix in a separate room	Potential for cross contamination does exist at AVC lab - the biosafety cabinet where extraction of strong positive control material takes place in the same biosafety cabinet with the same pipets as is used for adding template to PCR master mix
3. 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	Segment 8 -developed by DFO with a predicted 179 bp product according to the draft protocol. However, I checked and it's a 169 bp product. The product overlaps that of the one described in the 2009 Manual of Diagnostic Tests for Aquatic Animals	Both Segment 8 protocols should in theory be similar with respect to analytic sensitivity, however, we have no direct comparison data
4. 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	Two-step RT-PCR.; RNA normalized prior to carrying out the RT step with High Capcity cDNA Kit (ABI)	In theory and in practice a two-step RT-PCR is usually more sensitive than a one-step RT-PCR
5. PCR chemistry utilized	LC480® Master Hydrolysis Probe (Roche)	TaqMan Universal PCR Master Mix Kit (ABI)	None to minimal
6. Primer and probe concentrations	Calculations in Master mix worksheet are confusing and needed clarification (720 nM for primers and 200 nM for probe). The group was left with the impression that there was lack of understanding with respect to setting up PCR master mixes	480 nM for primers, 200 nM for probe	Primer and probe concentrations in combination with the chemistries used can affect analytic sensitivity
7. 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.	β-tubulin internal control was run with each assay as was measurement of OD_{260} and OD_{280} by MultiSkan to quantify the RNA. In addition, RNA integrity was assessed using the BIO-RAD Experion system. Results indicated RNA degradation in the samples received by DFO.	This could be significant since we have no indication of the quality of the samples that AVC got positive results for
8. Instrument platform used	Roche LC480	Stratagene Mx3000P	Instrument platforms and associated analysis software can have some affect on analytic sensitivty/specificity and result interpretation
9. Cycling conditions	63°C x 3 minutes; 95°C x 30 seconds; 45 cycles @ 95°C x 15 sec, 60°C x 1 min, 72°C x 1 sec	50°C x 2 minutes; 95°C x 10 minutes; 40 cycles @ 95°C x 30 sec, 60°C x 30 sec, 72°C x 30 sec	The brief RT step in the AVC protocol (3 minutes)may sacrifice sensitivity
10. 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.	Yes. Assay validated using infected and non-infected Atlantic Salmon kidney tissue	

11. Positive control	ISAV RNA extracted from TO cell culture lysates - Cps of positive controls are low (<20) which can be a source of contamination	*in vitro* transcribed RNA from plasmid DNA that contains a 26 bp insert	ISAV RNA is a potential source of cross-contamination. Furthermore it makes it distinguishing between true positives and contamination with positive control difficult.