A3.6 Polymerase Chain Reaction (PCR) Position Statements

A. 2000 – 2002 Position Statements

1. **Initial Position Statement**
   This chapter was designed to supplement references to molecular techniques referenced in earlier chapters. Included are general considerations for insuring that contamination does not occur and to insure the integrity of the assay. These general protocols that can be found in many general primers for PCR and are intended to provide background information for laboratories that are just setting up PCR diagnostics.

B. 2002 – 2003 Position Statements

No changes or review requested.

C. 2003 – 2004 Position Statements

The committee agreed to a suggestion to provide a comprehensive table outlining all of the PCR protocols.

The virology subcommittee contributed a new section to the PCR chapter. The paper used as the source of the LMBC PCR method also included an option to use Uracil N glycosylase (UNG) and dUPT to reduce carry-over contamination. The virology committee elected to include this method in the PCR chapter as an alternative that could be applied to PCR reaction in any laboratory where contamination is a problem, but felt that it was not reasonable to require UNG in all labs doing LMBV PCR.

D. 2006 – 2007 Position Statements

No changes or review requested.

E. 2008 – 2010 Position Statements

Updates were made to the PCR chapter to include information on quantitative PCR.

F. 2011-2012 Position Statements

No changes or review requested.

G. 2013 – 2014 Position Statements

No changes or review requested.

H. 2019-2020 Position Statements

1. **Should the Viral Hemorrhagic Septicemia Virus (VHSV) quantitative polymerase chain reaction (qPCR) assay (Jonstrup et al. 2013) be adopted into Section 2 (Aquatic Animal Health Inspections) of the American Fisheries Society Fish Health Section Blue Book**. This assay was evaluated for purpose by the USDA-NVSL (Warg et al. 2014a; Warg et al. 2014b).

2. **Should the *Renibacterium salmoninarum* (*R. sal.*) qPCR assay (Chase et al. 2006) be adopted into Section 2 (Aquatic Animal Health Inspections) of the American Fisheries Society Fish Health Section Blue Book.** This assay was designed to detect *R. sal.* DNA in fish tissues, ovarian fluid, or Bacterial isolates by the Western Fisheries Research Center.

a. Two sub-committees were assembled to evaluate the adoption of two real-time PCR assays described above. Both committees came to the conclusion that the adoption of these two validated and widely tested molecular assays into the Fish Health Section Blue Book would be a needed first step to bring the existing document up to date and relevant in the world of aquatic animal molecular diagnostics. The advantages of real-time PCR over conventional PCR are numerous; including: quantification in exponential growth phase, reduced contamination (closed-tube, no post-PCR processing), extra level of specificity with probe technology, and increased dynamic range. Standard Operating Procedures (SOPs) for each real-time PCR assay have been written in formal Washington Animal Disease Diagnostic Laboratory (WADDL) SOP format to initiate a consistent new methodology for new molecular assays assimilated into the Blue Book.

Note: Please refer to Appendix 3.3 Bacteriology Position Statements, subheading I. for further information concerning the adoption of the *Renibacterium salmoninarum* (*R. sal.*) qPCR assay (Chase et al. 2006).
Please refer to Appendix 3.4 Virology Position Statements, subheading I. for further information concerning the adoption of the Viral Hemorrhagic Septicemia Virus (VHSV) quantitative polymerase chain reaction (qPCR) assay (Jonstrup et al. 2013).