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Validation of Hydrogen Peroxide Dose Verification Field Method for Saltwater

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35% Perox-Aid® (35% hydrogen peroxide; H_2O_2) is an important drug used as an antimicrobial in aquaculture and is currently approved for the following uses: (A) control of mortality in freshwater-reared (1) finfish eggs due to saprolgeniasis, (2) salmonids due to bacterial gill disease, (3) cool- and warmwater finfish due to external columnaris disease, (4) coldwater finfish, fingerling and adult coolwater finfish, and fingerling and adult warmwater finfish due to saprolgeniasis, and (B) treatment and control of *Gyrodactylus* spp. in freshwater-reared salmonids. Virtually all efforts to date have been for freshwater use although 35% Perox Aid® is frequently used under the U.S. Fish and Wildlife Service's Investigational New Animal Drug (INAD) exemption in marine settings for parasite control, and most frequently when fish are in open ocean net-pens. Treatment of fish in net-pens presents considerable logistical challenges, including finding a method to measure H_2O_2 concentration and fate accurately, reproducibly, and rapidly in water samples on tender boats with limited laboratory space for such analysis and because of the lack of commercial probes that measure H_2O_2 at concentrations below 20 mg/L. As such, the H_2O_2 dose verification method that had been used for all freshwater testing was modified for field testing in a marine environment. These modifications included removing all glassware, pre aliquoting the reagents used in the titration, and utilizing 50 mL conical tubes and 20 mL syringes, making the procedure more compact, safer, and faster in a field situation.

Methods

The study was conducted at Blue Ocean Mariculture Nursery Facility, Kona, HI on Sept 10, 2024. Water samples were prepared and then measured to verify H_2O_2 concentrations following procedures described in AADAP SOP MISC 261. Four analysts were involved in the dose-verification study and each sample was analyzed in duplicate by two different analysts using randomization and single blinded analysis. The following nine calculated H_2O_2 concentrations were prepared: 50, 75, 100, 150, 200, 250, 300, 350, and 400 mg/L. The order that samples with different concentrations were analyzed was randomly assigned (SOP MISC 237) and analysts were blinded to the H_2O_2 concentration at the time of analysis. Small batches (1 L) of 35% Perox Aid® were made up with filtered seawater in 1 liter plastic Nalgene bottles simulating a netpen water sample. Samples were aliquoted to 50 mL conical tubes and titrated with 0.02 M potassium permanganate until a pale pink color is observed after addition of saturated manganese sulfate and 5.0 N sulfuric acid to the sample. Samples were diluted before titration based on the expected target H_2O_2 concentration as identified using MQUANT test strips, (i.e., 50-100 mg/L H_2O_2 diluted 2:3; 101-150 mg/L H_2O_2 diluted 1:4, and 151-400 mg/L H_2O_2 diluted 1:9). See AADAP SOP MISC 261 for a more detailed description of the analytical procedures.

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Results and Discussion

Overall, results show that the field titration method is accurate regardless of analyst (Figure 1). The mean ± SD percentage error was 7.9 ± 6.0% across all samples analyzed. This was well within the ±25% error described as acceptable in the pivotal protocols under which efficacy and target animal safety studies are conducted. One shortcoming of the titration method used is that the precision is limited. Each drop of $KMnO_4$ (25 µL) represents a specific amount of H_2O_2 that can be used up in the reaction. When measuring higher concentrations of H_2O_2, the precision of the titration method is reduced with increasing dilution of the sample. For example, at the lowest and highest H_2O_2 concentration range and the greatest dilution, the method can only determine the concentration to the nearest 2.1 and 8.5 mg/L H_2O_2, respectively. Once familiar with the SOP, analysts were able to titrate samples accurately and safely in less than one minute per sample.

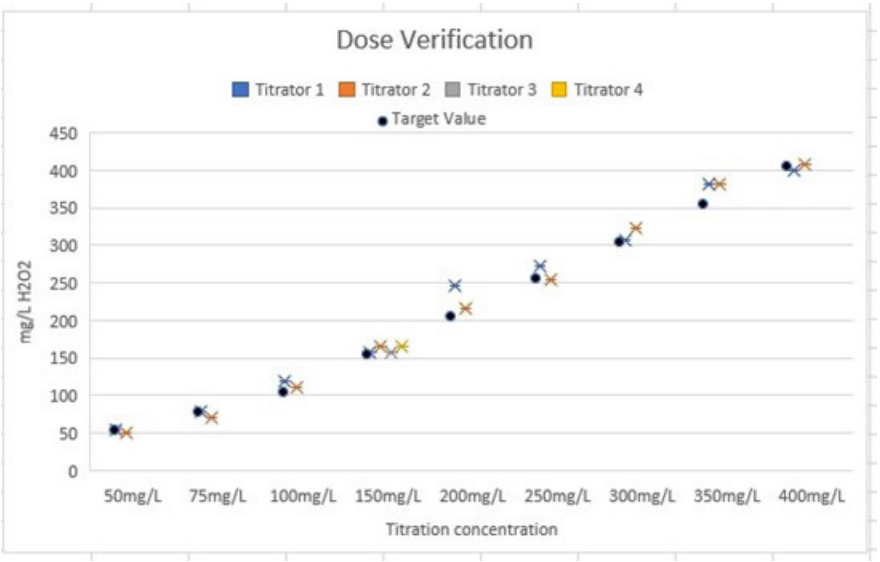

Figure 1. Analytically verified concentrations of H_2O_2 at each target concentration tested by two analysts.

Acknowledgments

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