

AQUATIZE®: Evaluation as Bacterial Disinfectant: AOAC Protocol 965.13

In 1996 and 1997, ABC Research (Gainesville, Florida) conducted two standardized studies following AOAC method 965.13 to evaluate Aquatize® as a disinfectant for bacterially contaminated water. Standard bacteriology cultivations were conducted on species-specific agar medium in petri plates and in tubes containing lactose broth. In the initial test, 500 ml flasks containing the appropriate dilution of the test material were inoculated with 1×10^6 CFU/ml of either *Escherichia coli* ATCC 11229 or *Enterococcus faecium* ATCC 6569. Dilutions of 1:2000 (A) and 1:5000 (B) of the test material were used. After adding the bacteria to the flasks, aliquots were removed at 0.5, 1, 2, 3, 4, 5, and 10 minutes and added to the plates and the tubes. Effectiveness of the test material was compared to 0.6 ppm sodium hypochlorite. In the second test, a single dilution of Aquatize® (1:5000) was evaluated for its efficacy at intervals of 5, 10, 15, 20, 25 and 20 minutes to disinfect water containing either *Shigella sonnei*, *Streptococcus faecalis*, *Listeria monocytogenes*, *E. coli*, *Salmonella typhimurium*, *Campylobacter jejuni*, *Streptococcus suis*, or *Pasteurella multocida*. The species *E. coli* 0157:H7, *Salmonella enteritidis* and *Pseudomonas aeruginosa* were tested at 1:2000 and 1:5000 dilutions of the test material. Each species was cultivated on species-specific medium according to ATCC recommendations. Protocol changes were approved in advance by the Chief of Efficacy and Science Support Branch, EPA. Three separate batches (1-3) of Aquatize® were evaluated.

In the first test, no growth was evident after 30 seconds exposure to sample A or the hypochlorite, but approximately 2 minutes exposure to sample B was required to completely stop growth. In the second test, exposure of an initial inoculum of 7.49 log units *E. coli* 0157:H7 to the 1:2000 dilution of the test material revealed that 2 of 3 batches of test material disinfected completely in 10 minutes whereas the other batch completed disinfection in 15 minutes. With an initial inoculum of 7.67 log units of *S. enteritidis*, batches 1 and 2 (1:2000) completed disinfection in 10 minutes and batch 3 (1:2000) completely disinfected the water in 5 minutes. At the 1:2000 dilution all three batches of the test material completely disinfected water inoculated with 7.14 log units of *P. aeruginosa* within 5 minutes. For the remaining microbial pathogens, exposure to a 1:5000 dilution of the all batches of test material revealed complete disinfection within 5 minutes.

Under the constraints of the standardized protocol, Aquatize® product performed as well as 0.6 ppm sodium hypochlorite at the 1:2000 in the first test against *E. coli* and *E. faecium*. In the second test, exposure of *E. coli* 0157:H7 and *S. enteritidis* to 1:2000 Aquatize® resulted in disinfection at 10-15 minutes but 0.6 ppm hypochlorite disinfected the water at 5 minutes. Disinfection of *P. aeruginosa* at 1:2000 Aquatize® equaled 0.6 ppm hypochlorite. Disinfection of all other pathogenic species with 1:5000 Aquatize® was equal to disinfection with 0.6 ppm hypochlorite. In conclusion, Aquatize® appears to be an acceptable disinfectant for water contaminated with any of the species listed herein.