Efficacy of Nucleic Acid Probes for Detection of Poliovirus in Water Disinfected by Chlorine, Chlorine Dioxide, Ozone, and UV Radiation

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MilliQ water was inoculated with poliovirus type 1 strain LSc-1 and was treated with disinfectants, including chlorine, chlorine dioxide, ozone, and UV light. No relationship between probes and plaque assays were seen, demonstrating that viral nucleic acids were not destroyed. These findings suggest that nucleic acid probes cannot distinguish between infectious and noninfectious viruses and cannot be used in the evaluation of treated waters.

Water is mandated to be free of pathogenic bacteria, protozoa, and viruses so as not to pose a health threat. The standard method for monitoring viruses in the environment has been by cell culturing. This assay is sensitive enough to detect as little as 1 infectious unit but has many drawbacks, including the lack of a universal cell line to detect all enteric viruses, the inability of some viruses to exhibit cytopathic effects, and time delays of up to 4 weeks (5).

Molecular techniques such as PCR and nucleic acid probe assays can give results within 24 h, and these have been shown to be more sensitive for virus detection than cell culturing in detecting viruses from environmental samples (6). However, one potential problem associated with nonculture assays is the inability to differentiate infectious from noninfectious viruses. While disinfection may disrupt the viral protein coat, rendering the virus noninfectious, it may not destroy the nucleic acid, which could still be detected, rendering a false-positive result. This problem would limit the application of nucleic acid probes and, potentially, of PCR assays to nondisinfected waters, such as groundwater and surface source waters.

Each disinfectant destroys viruses by a different mechanism and has a variety of advantages and disadvantages in its use to treat water. Chlorine is the least popular method of disinfection because of its low cost and ease of use. However, high levels of chlorine can lead to the production of trihalomethanes, such as chloroform, which are potentially carcinogenic (5). Chlorine dioxide has been proposed as an alternative. Water treated with chlorine dioxide has a better taste, and the chlorine dioxide can easily be removed by aeration and does not lead to the production of trihalomethanes. Its bactericidal efficiency is not as dependent on the pH of the water, and it has a superior viricidal activity (9). Previous information suggests that the viricidal efficiency of chlorine dioxide increases with pH (1).

During UV radiation, organic molecules absorb the energy. This energy is then dissipated, disrupting unsaturated bonds. Increases in the length of exposure, the intensity of the light, and the proximity of the radiation source all have a direct correlation with virucidal activity (4). Ozone is a powerful oxidant. It attacks organic compounds by adding oxygen atoms to the unsaturated carbon-carbon bonds, yielding carboxylic acids, ketones, and aldehydes (2).

MilliQ water at room temperature was brought to a pH of 6, 7, or 9 using the buffers 500 mM KH2PO4 (pH 4.4) and 500 mM Na2HPO4 (pH 9.9). The water was spiked with chlorine to give a final residual of 1 mg/liter. Chlorine residuals were read by mixing an aliquot of water with AccuVac DPD Free Chlorine Reagent (Hach, Loveland, Colo.) and read in a DR/100 Direct Reading Spectrophotometer (Hach). Forty-five milliliters of chlorinated water was added to four separate 50-ml polyethylene tubes, and then 500 μl of the poliovirus dilution was added. The four time points of disinfection tested were 0, 2, 10, and 60 min. The reaction was stopped at the appropriate time by taking two 10-ml aliquots and dispensing them into two 15-ml conical polyethylene tubes containing 500 μl of 0.05 M Na2S2O3. To ensure no time delay for the 0-min time point, 2.25 ml of Na2S2O3 was added to the chlorinated water before the virus was added, and then the sample was aliquoted into the tubes. Chlorine residuals were measured at each time point to ensure that residual chlorine with a concentration of 0.2 mg/liter was still available for virus inactivation. All samples were returned to pH 7 when the run was complete. Chlorine dioxide was similarly assayed. An initial dose of 1 mg of chlorine dioxide per liter was used, and then the same pH and time parameters set in the chlorine trial were used. Residuals were measured in a DR/2000 Direct Reading Spectrophotometer.

For the ozone study, 300 ml of distilled water containing poliovirus was put into a 500-ml Pyrex glass bubbler. A 30-ml aliquot was taken at time zero. Ozone (1%) was then bubbled through the solution at a rate of 1 liter/min. An Orec Ozonator (Ozone Research & Equipment Corporation, Phoenix, Ariz.) set at 0.2 A was used to generate the ozone. Aliquots were taken at 0, 2, 10, and 60 min.

In the UV study, 5 ml of distilled water containing poliovirus was placed in sterile glass petri dishes. The depth of the water did not exceed 5 mm. Samples were placed under a UV light source at a distance to give 5 mW/cm². The intensity was measured using a Blak-Ray UV meter (Ultra-Violet Products, Inc., San Gabriel, Calif.). Aliquots were taken at time zero and at 2, 10, and 60 min.

Each study was performed minimally in triplicate, and the

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titer of poliovirus at each time point was assayed. To determine the viral titer of each sample, 100-μl dilutions of 10^6 to 10^-3 were taken from each sample and allowed to infect 25-cm² flasks of buffalo green monkey (BGM) cells according to the methods described by Dahling and Wright (3). Plaques were counted after 48 h.

A second aliquot of each sample was assayed by digoxigenin-labeled probe. The nucleic acid probe was generated from the plasmid pVR104, which contains poliovirus cDNA. A 149-bp piece from the 5' noncoding region was amplified by PCR using a digoxigenin DNA labeling mixture (Boehringer Mannheim) instead of deoxyxynucleoside triphosphates. The sensitivity for this assay was determined to be 0.1 pg of cDNA or 9 × 10^2 PFU of poliovirus type 1 strain LSc-1 (6).

Five hundred microliters of each disinfection sample was incubated at 55°C for 1 h with a 400-μg/ml final concentration of proteinase K. Each sample was serially diluted in diethyl pyrocarbonate-treated water (8) to 10^-3. Five hundred microliters of each dilution was spotted onto GeneScreen nylon-backed membranes (DuPont, Boston, Mass.) with a dot blot apparatus. Negative controls consisted of spotting 500 μl of proteinase K-treated water which had not been inoculated with virus prior to the disinfection treatment. Viral nucleic acid was detected by the colorimetric digoxigenin probe procedure (7), with the following exceptions: the hybridization solution contained 3% sodium dodecyl sulfate, and the preblocking wash before addition of the antibody contained 0.3% Tween 20.

Table 1 presents the findings for one study with chlorine and chlorine dioxide. The other trials had comparable results. A two-log reduction by plaque assays was seen at both pH 6 and pH 7 in the chlorine-disinfected samples. No infectious viruses were present in either sample within 10 min. The signal intensity of the nucleic acid probe did not decrease during the hour period, starting with an initial sensitivity of 50 PFU. At pH 9, all viruses were inactivated in 2 min. An initial reduction in probe signal intensity was seen throughout the pH 9 analysis even when Na2S2O3 was previously added. Chlorine dioxide-disinfected samples had similar results, including the signal reduction with nucleic acid probes at pH 9.

Table 2 presents the findings for one study with ozone and UV light. The other trials had comparable results. In the ozone-treated samples, the number of infectious viruses was reduced by two-thirds within 2 min. No infectious viruses were present by 10 min. In the UV-irradiated samples, a three-log reduction had occurred within 2 min. All viruses were inactivated by 60 min. No reduction in signal in either ozone- or UV-treated samples was seen with nucleic acid probes.

No correlation between plaque assays and nucleic acid probes was seen for any disinfectant. Each disinfectant was shown to be adequate in reducing virus infectivity; however, none reduced the amount of viral nucleic acid within 1 h. The only possible virus degradation seen was due to an exposure to pH 9. The other alternative is that a by-product may lower the sensitivity of the probe assay without disrupting virus infectivity.

This study shows that probe assays on nylon membranes cannot distinguish between infectious and disinfected viruses. Nylon membrane nucleic acid probe assays are not good candidates for sole monitoring of virus in treated waters. Other nucleic acid detection assays, such as PCR, may also not be able to discern virus infectivity. An in situ probe assay or in situ PCR could ameliorate this problem by detecting only viral nucleic acid that is replicating inside a cell.

Because of their cheaper cost and shorter time, nucleic acid probe assays on nylon membranes can be used as a technique for rapid screening of treated waters. If the result is negative, the water has been adequately processed, and if the result is positive, the water must be further analyzed by cell culture. Nucleic acid probing is the assay of choice to monitor source waters which are to be used for drinking, because potable water should be free of treatment plant effluent or other sewage discharges. Any enteric viral nucleic acid discovered, whether infectious or noninfectious, can be deemed a health threat, because it indicates a fecal contamination. A closer correlation between nucleic acid probe assays and cell cultures may be seen in environmental samples containing a microbial flora. Organisms can take up nucleic acids directly or produce compounds, such as ammonia, which can have a virucidal activity (8).

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