Impermeability of PETG Bottles to Hydrogen Peroxide Sterilants

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Key Words
PETG bottles, vaporized hydrogen peroxide, hydrogen peroxide, sterilization, clean rooms.

Introduction
Hydrogen peroxide (H$_2$O$_2$) is a highly effective antimicrobial agent. Due to this property, and its availability and relatively low cost, it is commonly used as a sterilant in many areas where microbial contamination must be reduced or eliminated. Liquid H$_2$O$_2$ solutions have long been used to sterilize materials or equipment entering a “clean” area in laboratory cell culture operations, small and large scale bioproduction, and in food production. Increasingly, vaporized hydrogen peroxide (VHP) systems have been used to sterilize materials entering “clean” areas, especially those entering through pass-through isolators. Peroxide residues possibly left behind from such procedures, however, could affect sensitive systems that the materials are used in. For example, peroxide residue remaining on materials used in cell culture would adversely affect the growth of cells grown on those materials.

Thermo Scientific Nalgene PETG Sterile Square Media Bottles are often used for many different applications, including storage of component materials for cell culture systems. The best way to guarantee there is no H$_2$O$_2$ residue in the bottles after the sterilization process, is to ensure that no H$_2$O$_2$ enters the bottle in the first place. Since these bottles can be shipped presterilized, exposure of the inside of the bottle to H$_2$O$_2$ is not necessary. Here, we verify that H$_2$O$_2$ does not enter PETG bottles through spray-on applications of hydrogen peroxide liquid solution or through a VHP exposure cycle in an isolator.

Materials and Equipment
- 125 mL Nalgene PETG Sterile Square Media Bottles (Thermo Fisher Scientific 2019-0125)
- Steri-Perox 6% Hydrogen Peroxide Solution (Veltek Associates Inc.)
- Steraffirm Vapor Process Indicator Strips (STERIS PCC051 and NB305)
- VHP Generator (STERIS)
- Hydrogen Peroxide Vapor Monitor (Guided Wave)
Methods
Two bottle types were chosen to represent the range of sizes and different closures used on Nalgene™ PETG Sterile Square Media Bottles. VHP indicator strips were placed in each bottle (Figure 1), and bottles were capped with the recommended torque specification.

VHP Exposure
Bottles containing both PCC051 and NB305 indicator strips were sent to a third-party testing laboratory for analysis. For each size bottle, N=4 experimental bottles were used, as well as N=1 negative control (not exposed to VHP) and N=1 positive control (the seal was compromised with a hole in the closure to provide VHP exposure inside the bottle). The VHP generator was set up to provide a one-hour exposure at 2g/min injection rate. This resulted in a VHP concentration of approximately 1500 ppm inside the isolator for the one-hour exposure cycle as measured by the Guided Wave hydrogen peroxide vapor monitor (HPVM). In the latter part of the cycle the HPVM indicated that condensation was present inside the chamber.

At the conclusion of the cycle, a reading was taken inside the isolator which indicated that greater than 20 ppm VHP concentration was still present (20 ppm was the upper limit of the hand-held VHP monitor used). A 6-hour aeration cycle was then started. At the end of the 6-hour aeration, approximately 2 ppm concentration was still present in the isolator. The specimens were removed and exposure indicator strips were observed at that time.

Spray application of liquid H₂O₂ solution
Bottles containing PCC501 indicator strips were placed in a plastic bin. For each size bottle, N=4 experimental bottles, as well as N=1 positive control (with compromised seal) and N=1 negative control (left out of the bin and not sprayed) were used. All bottles were sprayed with a heavy application of Steri-Perox 6% hydrogen peroxide solution. Two indicator strips were taped to the underside of the bin lid, with no direct contact with peroxide solution, and the lid was closed.

Results and discussion

VHP Exposure
Exposure indicator strips in all experimental bottles showed no change after a VHP exposure cycle. The 2 L positive control bottle also showed no color change. Only the exposure indicator in the 125 mL positive control bottle showed any color change after the VHP exposure cycle.

Spray application of liquid H₂O₂ solution
Exposure indicator strips in all experimental bottles showed no change after overnight application of H₂O₂ solution. Indicator strips attached to the bin lid showed complete color change after overnight exposure. Similar to the VHP results, no color change was visible in the 2 L positive control bottle, while a slight color change (not as complete as the strips from the lid) was visible in the 125 mL positive control bottle.

These results indicate that Nalgene PETG media bottles, when properly closed, are impermeable to hydrogen peroxide under sterilization procedures normally used to pass materials into clean areas. While the indicator strips in large positive control bottles failed to change, the exposure in small bottles indicates that some peroxide vapor makes it into these bottles through the compromised closure.

We therefore recommend that bottles are properly closed with adequate torque applied in situations where it is critical to keep H₂O₂ outside the bottle.

Conclusion
Nalgene PETG Sterile Square Media Bottles provide a great solution as a bottle for use in hydrogen peroxide sterilized systems where peroxide must not permeate the inside of the bottle.