Impermeability of PETG bottles to hydrogen peroxide sterilants

Introduction

Hydrogen peroxide (H_2O_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_2O_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 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 residue 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™ Polyethylene Terephthalate Glycol (PETG) Sterile Square Media Bottles are used for many different applications, including storage of component materials for cell culture systems. The best way to guarantee there is no H_2O_2 residue in the bottles after the sterilization process is to ensure that no H_2O_2 enters the bottle in the first place. Since these bottles can be shipped presterilized, exposure of the inside of the bottle to H_2O_2 by the user is not necessary. Here, we verify that H_2O_2 does not enter these PETG bottles through spray-on application of H_2O_2 liquid solution or through a VHP exposure cycle in an isolator.

Figure 1. VHP process indicator strips inside 125 mL media bottles.

Materials and equipment

- 2 L Nalgene PETG Sterile Square Media Bottles (Thermo Fisher Scientific, Cat. No. 2019-2000)
- 125 mL Nalgene PETG Sterile Square Media Bottles (Thermo Fisher Scientific, Cat. No. 2019-0125)
- Steri-Perox™ 6% Hydrogen Peroxide Solution (Veltek Associates Inc.)
- Steraffirm™ Vapor Process Indicator Strips (STERIS, Cat. No. PCC051 and NB305)
- VHP Generator (STERIS)
- Hydrogen Peroxide Vapor Monitor (Guided Wave)

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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 Cat. No. PCC051 and NB305 indicator strips were sent to a third-party testing laboratory for analysis. For each bottle size, 4 experimental bottles were used, as well as 1 negative control (not exposed to VHP) and 1 positive control (the seal was compromised with a hole in the closure to allow VHP into the bottle). The VHP generator was set up to provide a 1 hr exposure at an injection rate of 2 g/min. This resulted in a VHP concentration of approximately 1,500 ppm inside the isolator for the 1 hr exposure cycle, as measured by the 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 indicating that a concentration of >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 was still present in the isolator. The bottles were removed and the exposure indicator strips were observed at that time.

Spray application of liquid H_2O_2 solution

Bottles containing Cat. No. PCC501 indicator strips were placed in a plastic bin. For each bottle size, 4 experimental bottles, as well as 1 positive control (with compromised seal) and 1 negative control (left out of the bin and not sprayed) were used. All bottles were sprayed heavily with of Steri-Perox 6% Hydrogen Peroxide Solution. Two indicator strips were taped to the underside of the bin's lid, with no direct contact with the peroxide solution, and the bin was closed.

The bin was left overnight to mimic a common procedure used to pass materials into clean areas. After overnight exposure, the bottles were removed from the bin and all exposure strips were observed.

Results

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 indicator strip in the 125 mL positive control bottle showed any color change after the VHP exposure cycle.

Spray application of liquid H_2O_2 solution

Exposure indicator strips in all experimental bottles showed no change overnight after application of H_2O_2 solution. Indicator strips attached to the bin's lid showed a 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 bin's lid) was visible in the 125 mL positive control bottle. These results indicate that Nalgene PETG media bottles, when properly closed, are impermeable to H_2O_2 under sterilization procedures normally used to pass materials into clean areas. While the indicator strips in the large positive control bottles failed to change, the exposure detected in the small bottles indicates that some peroxide vapor makes it into these bottles through the compromised closure. We therefore recommend that bottles be properly closed, with adequate torque applied, when it is critical to keep H_2O_2 out.

Conclusion

Nalgene PETG Sterile Square Media Bottles provide an excellent solution as a bottle for use in H_2O_2-sterilized systems where peroxide must not permeate the bottle.

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