Nalgene PETG bottle performance at –70°C

Keywords
Nalgene, PETG, ULT freezer, cold storage

Abstract

The purpose of this study is to extend the recommended temperature range for Thermo Scientific™ Nalgene™ PETG containers from –40°C to as low as –70°C. Back-off torque studies and pressure leak testing were performed on 1 L and 2 L bottles at –70°C and –40°C. Additionally, impact fracture studies were performed on bottles sterilized with two different sterilization doses, frozen at –70°C and then transferred to –40°C to mimic typical use. Torque degradation was greater at –70°C in comparison to –40°C during back-off torque studies for both 1 L and 2 L bottle sizes. However, pressure leak testing indicated that closure seal integrity was intact at both temperatures. Both bottle sizes survived impact at the equilibrated –40°C after –70°C storage. The use of Thermo Scientific™ Nalgene™ PETG bottles at –70°C is recommended based on the conditions evaluated; testing in actual use conditions is also recommended as results may vary depending upon application.

Background

Polyethylene terephthalate glycol (PETG) is a type of thermoplastic polyester. A thermoplastic is a polymer that becomes soft and pliable when heated without a change in its intrinsic properties. With these physical characteristics, PETG has been a commonly used material because it is a high-performance plastic that is lightweight with good impact strength, and can be used in a wide range of temperatures. PETG is also optically clear and BPA- and animal origin–free. PETG bottles are currently not recommended for use below –40°C. The purpose of this study is to extend the recommended temperature range for existing Nalgene PETG containers from –40°C to as low as –70°C. Here, we discuss the factors to consider when evaluating containers for sample leakage and loss.

Important considerations

Fill volume

Containers should not be filled with a volume greater than the recommended capacity. Additionally, bottles require adequate space during freezing to allow appropriate cooling of the fluid inside the container. Otherwise, the container may show evidence of cracking and/or crazing.

Cracking and crazing

A crack is the separation of an object or material into two or more pieces as a result of applied stress, resulting in leakage of the fluid from the container. Conversely, crazing is a cosmetic observance that does not show evidence of leaking. Crazing occurs at stressed regions and propagates perpendicular to the applied tension.

Sterilization by gamma-irradiation

Experimental samples should be irradiated to at least the highest dose that will be encountered during routine processing. A preferred and more conservative method is to irradiate samples at twice the anticipated maximum dose [1]. It is expected that bottles evaluated with a higher irradiation dose would have a higher failure rate because plastics subjected to irradiation during sterilization results in changes in the polymer structure. Irradiation creates free radicals that recombine to form crosslinks.

Crosslinking impedes the molecular movement of the polymer, resulting in an increase in thermal resistance and the improvement of mechanical strength and chemical resistance. Conversely, with chain scissioning, the polymer chains are broken and molecular mass decreases. Scissioning and crosslinking occur at the same time where one may predominate over the other, depending upon the polymer and the irradiation dose [2].

Torque specifications

Nalgene bottles have minimum and maximum applied torque specifications. The applied torque is the rotational force in which a closure is applied to a container. Applied torque affects the seal integrity of the bottle and the closure. Back-off torque is the rotational force necessary to open, loosen, or remove the closure. Torque degradation is the relationship between applied and back-off torque and establishes the sealing characteristics of the bottle and closure assembly. When evaluating samples, it is recommended that the minimum applied torque specifications are used in order to establish worst-case conditions in testing.

Pressure leak testing

After filling bottles to the nominal capacity and applying the closure to the proper torque specification, the ability to detect leaks is necessary to ensure the seal integrity of bottle and closure assemblies. A pressure apparatus can be inserted into the container in order to pressurize the container with a specified amount of pressure for a defined period of time so that fluid leakage can be visualized. This is a routine quality control test performed on various Nalgene bottles.

Table 1. Materials used in this study.

Description	Cat. No.
Nalgene Square Media Bottles	
PETG with HDPE closures, in shrink-wrapped trays, sterile, 1,000 mL, 38-430 closure, tray pack	342020-1000
PETG with HDPE closures, in shrink-wrapped trays, non-sterile, 1,000 mL, 38-430 closure, tray pack,	322020-1000
PETG with HDPE closures, in shrink-wrapped trays, sterile, 2,000 mL, 53B closure, tray pack	342020-2000
Chemicals	
Fisher Chemical™ Sodium Chloride	S271-1
Fisher Chemical™ Dextrose	D16-1
Environmental chambers	
Transformer (Acme Electric)	T-1-81052
Syle SR Micro Tenn™ II (Tenney Engineering Inc.)	T30R3
Other	
Hydraulic drop testing apparatus (Lansmont Corporation)	
Temperature data logger, USB-2416 (Measurement Computing Corporation)	
MCC DAQ Software Version 6.0 (Measurement Computing Corporation)	
Torque wrench and torque wrench adapters	

Methods

Visual observations

For each experiment, bottles were visually observed for physical deformities, including cracking or crazing.

Time to reach desired temperature

Containers were filled to the recommended capacity with a solution containing 1 g/L dextrose (glucose) and 9 g/L NaCl in water to mimic typical physiological conditions. Thermocouple wires were inserted through holes drilled in each closure and connected to a computer data logger. One thermocouple wire remained at room temperature as a control. Additionally, the frozen drop testing bottles were placed in the freezer in order to ensure that bottles used for testing reached the appropriate temperature (15x 1 L bottles and 15x 2 L bottles). Samples were placed in the –70°C freezer with adequate space and were arranged to prevent any location-based temperature bias. Once the –70°C temperature was obtained, the bottles were transferred to a –40°C freezer. Temperature data were collected every 10 seconds and the average of every 50 temperature readings was calculated. The data were normalized to 0°C, determined by the stabilization of temperature during the freezing phase in each container.

Impact fracture studies

1 L (n = 30) and 2 L (n = 30) bottles from inventory with the nominal sterilization dose were filled with test solution and were arranged in the –70°C freezer as previously described. After sufficient time for individual samples to reach –70°C and then –40°C, the samples were removed and were drop tested using a hydraulic drop test apparatus set at a height of 36 inches. The bottles were evaluated for breakage. After each impact fracture study, samples were examined for wall failures or deformities. Solutions were allowed to thaw and samples were examined for any solution leakage. 1 L Nalgene PETG bottles were sent to an external vendor for sterilization at two times the normal sterilization dose and were evaluated in the same manner (n = 30).

Freeze–thaw back-off torque degradation studies

Bottles from inventory were filled with test solution as previously described. Thirty samples of each bottle size at each temperature were tested. Closures were applied using a manual torque wrench to a specification of 27 in·lb (1 L bottles) or 38 in·lb (2 L bottles). Closure torque specifications were chosen based on the minimum quality control torque specifications for the bottles. After one freeze–thaw cycle, the same torque wrench was used to measure the maximum torque applied to remove the closure. The results for each condition were averaged and were reported as the decrease in torque as a percent of applied torque.

Pressure leak testing

Briefly, containers were filled and closures were applied using a manual torque wrench as described previously. 30 samples of each bottle size (1 L and 2 L) at each temperature (–70°C and –40°C) were evaluated after one freeze–thaw cycle. A pressure apparatus was inserted into the container and the 2 L and 1 L containers were pressurized for 2 minutes at 2 or 10 psi, respectively. A container was considered a failure if water escaped the closure.

Results

Visual observances

For each experiment, no bottles showed any evidence of deformity, cracking, or crazing.

Time to reach desired temperature

During –70°C storage, 1 L bottles were able to reach 0°C and cooled to –70°C slightly faster in comparison to the 2 L bottles, as expected. All bottles evaluated reached a final temperature of –70°C. One of the 2 L bottles required approximately 10 hours and 30 minutes to reach the desired temperature, which was the longest time observed (Figure 1). The bottles were transferred to a –40°C freezer and were continuously monitored with thermocouples. All bottles evaluated required approximately 2 hr 30 min to reach a temperature of –40°C (Figure 2). The times required to freeze were used as guidelines for future studies.

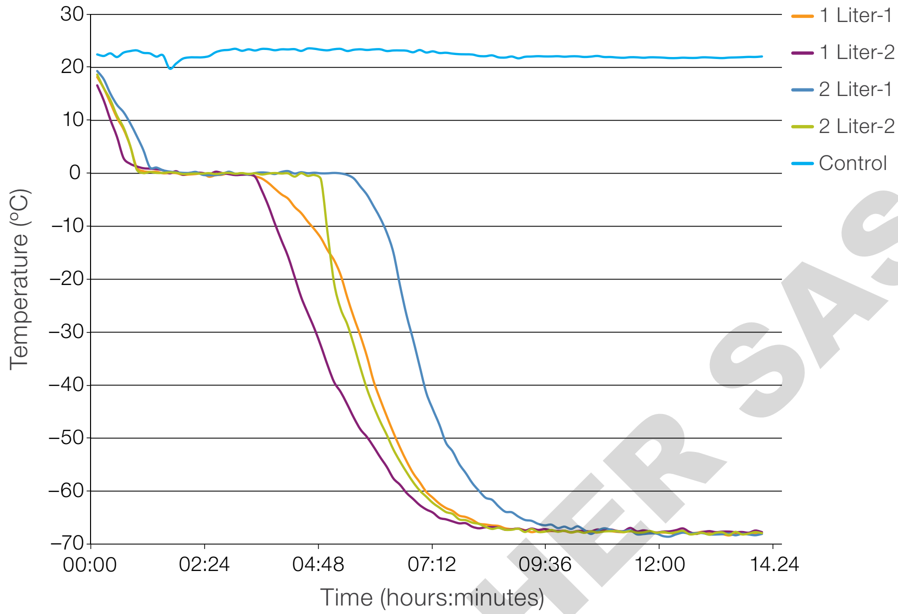

Figure 1. Averages of every 50 temperature readings over time for 1 L and 2 L Nalgene PETG bottles in a –70°C freezer.

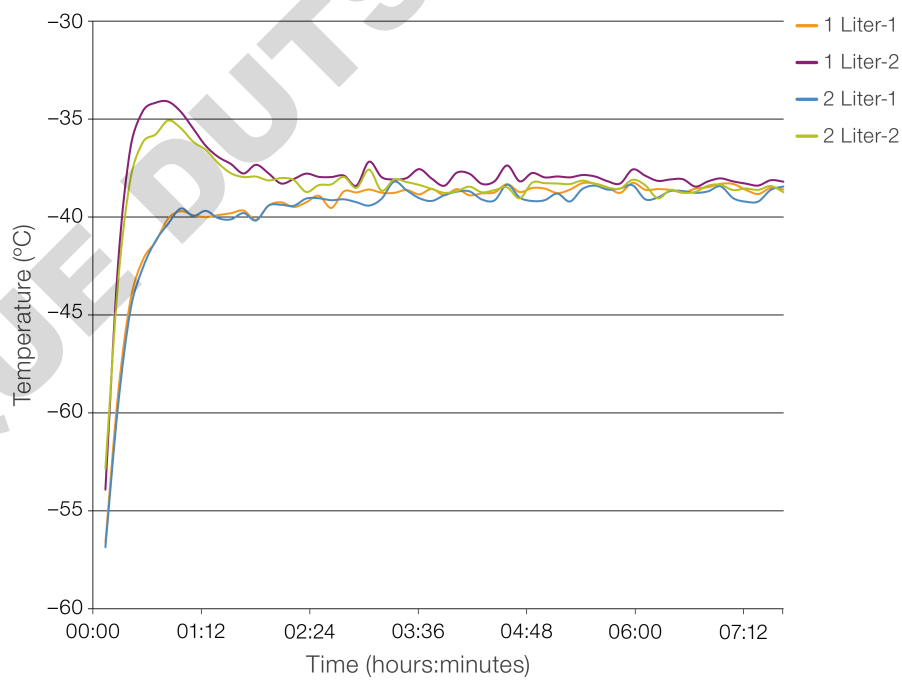

Figure 2. Averages of every 50 temperature readings over time for 1 L and 2 L Nalgene PETG bottles transferred from a –70°C freezer to –40°C freezer.

Impact fracture studies

None of the containers showed evidence of breakage or physical deformities at the nominal sterilization dose.

Two 1 L bottles containing twice the normal radiation dose (actual: 46–50 kGy) failed this test resulting in a 6.67% failure rate (Table 1). It is expected that bottles evaluated with a higher irradiation dose would have a higher failure rate, because plastics subjected to irradiation during sterilization acquire changes in polymer structure.

Freeze–thaw back-off torque degradation studies

1 L bottles showed 4.69% increased torque degradation and 2 L bottles showed 7.79% increased torque degradation after one freeze–thaw cycle at –70°C in comparison to one freeze–thaw cycle at –40°C (Table 2).

Pressure leak testing

After one freeze–thaw cycle at –40°C and –70°C, all 1 L bottles passed pressure leak tests at 10 psi for 2 minutes and all 2 L bottles passed pressure leak tests at 2 psi for 2 minutes (Table 3).

Discussion

The purpose of this study was to extend the recommended temperature range for existing Nalgene PETG containers from –40°C to as low as –70°C. In general, the performance of the two container sizes at the temperatures evaluated are comparable in the tests conducted.

Time to reach desired temperature

During initial testing, bottles were monitored for time to reach the appropriate temperature. These results were used as guidelines for testing that followed to ensure that the proper temperature was obtained.

Impact fracture studies

No failure was observed for either bottle size in frozen drop testing studies with nominal sterilization conditions when equilibrated to –40°C. 1 L bottles were drop tested with twice the normal sterilization dose, which is recommended for worst-case conditions, resulting in two failures (6.67% failure rate) when equilibrated to –40°C. It is expected that bottles evaluated with a higher irradiation dose would have a higher failure rate because plastics subjected to irradiation during sterilization will acquire changes in polymer structure. The risk here is minimal since customers typically will not encounter a need for such high sterilization dose.

Freeze–thaw back-off torque degradation and pressure leak testing

While 1 L and 2 L Nalgene PETG bottles showed better retention of closure torque after one freeze–thaw cycle at –40°C in comparison to –70°C, all bottles passed the pressure leak testing, indicating that closure seal integrity was maintained.

Table 1. Impact fracture study results of Nalgene PETG bottles stored at –70°C and then equilibrated to –40°C.*

Test condition	Sterilization dose	Sample size (n)	Number of failures	Failure rate (%)
1 L PETG bottle	Nominal	30	0	0
	Double	30	2	6.67
2 L PETG bottle	Nominal	30	0	0

* Upon immediate removal from –70°C storage, bottles have a high likelihood of fracture when dropped.

Table 2. Back-off torque results, shown as average actual torque required and as a percentage of the applied closure torque.

Test condition		Sample size (n)	Applied torque (in·lb)	Average back-off torque (in·lb)	Standard deviation	Torque degradation (% of applied torque)
–40°C	1 L PETG bottle	30	27	16.8	3.27	62.10
	2 L PETG bottle	30	38	21.7	3.13	57.19
–70°C	1 L PETG bottle	30	27	18.0	3.61	66.79
	2 L PETG bottle	29**	38	24.7	3.61	64.97

** For one sample, back-off torque was greater than the applied torque. This sample was removed from analysis.

Table 3. Pressure leak testing results.

Test condition		Pressure condition	Sample size (n)	Number of failures	Failure rate (%)
–40°C	1 L PETG bottle	2 min, 10 psi	30	0	0
	2 L PETG bottle	2 min, 2 psi	30	0	0
–70°C	1 L PETG bottle	2 min, 10 psi	30	0	0
	2 L PETG bottle	2 min, 2 psi	30	0	0

Conclusions

- Both 1 L and 2 L Nalgene PETG bottles performed comparably under simulated customer conditions (impact fracture studies).
- Both 1 L and 2 L Nalgene PETG bottles performed slightly better at –40°C in comparison to –70°C during back-off torque studies, and no bottles leaked during pressure leak testing, indicating that closure seal integrity was maintained.
- The use of Nalgene PETG bottles at –70°C is recommended based on the conditions evaluated; testing in actual use conditions is also recommended as results may vary depending upon application.

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Note: While the TSX Universal Series ULT Freezers were not utilized in this study of Nalgene PETG bottle performance at –70°C, they are part of the product offerings provided by Thermo Fisher Scientific.

Authors

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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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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_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 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_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 is not necessary. Here, we verify that H_2O_2 does not enter PETG bottles through spray-on applications of hydrogen peroxide 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 liter Nalgene PETG Sterile Square Media Bottles (Thermo Fisher Scientific 2019-2000)

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 H_2O_2 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. 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 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 H_2O_2 solution

Exposure indicator strips in all experimental bottles showed no change after overnight application of H_2O_2 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 H_2O_2 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.

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