

## Design &amp; Innovation

# Thermo Scientific Nalgene PETG bottle performance at -70°C

**Author**

Stephanie M. Carter and Joseph Granchelli, Application Laboratory, Thermo Fisher Scientific, Rochester, New York.

**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 1L and 2L 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 1L and 2L 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 PETG bottles at -70°C is recommended based on the conditions evaluated.

**Background**

Polyethylene Terephthalate G Copolyester (PETG) is a polyester thermoplastic polymer. A thermoplastic is a polymer that becomes soft and pliable when heated, without a change in its intrinsic properties. Due to these physical characteristics, PETG has been commonly used because it is a high performance plastic that is also lightweight, with good impact strength, and it can be used in a wide range of temperatures; it's also optically-clear and BPA- and animal-origin-free. PETG bottles are not currently recommended for use below -40°C. The purpose of this study is to extend the recommended temperature range for existing 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 of the vessel. Additionally, during freezing bottles require adequate space around vessels 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<sup>1</sup>. 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 which 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 dose<sup>2</sup>.

### 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

Description	Cat. No.
<b>Nalgene Square Media Bottles</b>	
PETG with HDPE closures, in shrink wrapped trays, sterile 1000mL, 38-430 closure, tray pack	342020-1000
PETG with HDPE closures, in shrink wrapped trays, non-sterile 1000mL, 38-430 closure, tray pack,	322020-1000
PETG with HDPE closures, in shrink wrapped trays, sterile 2000mL, 53B closure, tray pack	342020-2000
<b>Chemicals</b>	
Fisher Chemical™ NaCl	S271-1
Fisher Chemical Dextrose	D16-1
<b>Environmental chambers</b>	
Acme™ Transformer, Cat. No.	T-1-81052
Syle SR Micro Tenn™ II, Tenney™ Eng Inc.	T30R3
Fisher Chemical™ NaCl	S271-1
<b>Others</b>	
Hydraulic Drop Testing Apparatus – Lansmont™ Corporation	
Temperature Data Logger – Measurement Computing™ USB-2416	
MCC DAQ Software Version 6.0	
Torque wrench and torque wrench adaptors	

## 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 (15-1L bottles and 15-2L 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 was collected every 10 seconds and the average of every 50 temperature readings was calculated. The data was normalized to 0°C, determined by the stabilization of temperature during the freezing phase in each container.

### Impact fracture studies

1L (n=30) and 2L (n=30) samples 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. 1L 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

Samples 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-lbs (1L bottles) or 38 in-lbs (2L 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 (1L and 2L) 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 2L and 1L containers were pressurized at 2 or 10 psi for 2 minutes, 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, 1L bottles were able to reach 0°C and cooled to -70°C slightly faster to in comparison to the 2L bottles as expected. All bottles evaluated reached a final temperature of -70°C. One of the 2L bottles required approximately 10 hours and 30 minutes to obtain the desired temperature, which was the longest time observed (Figure 1). The bottles were transferred to -40°C and were continuously monitored with thermocouples. All bottles evaluated required approximately 2 hours 30 minutes to obtain a temperature of -40°C (Figure 2). The time required to freeze were used as guidelines for future studies.

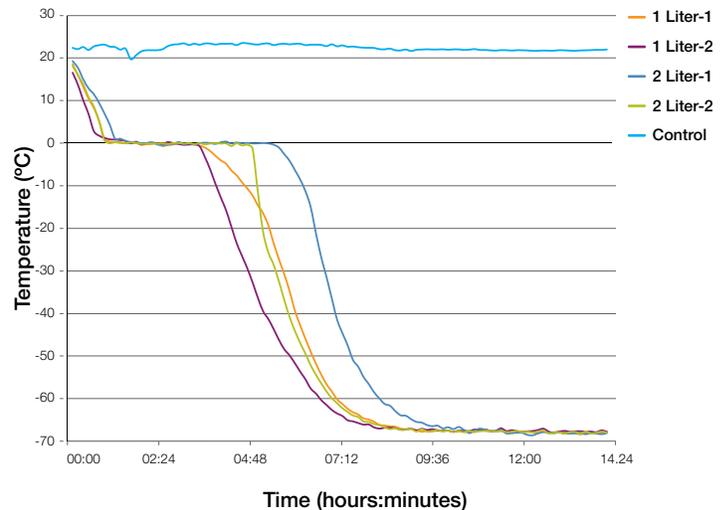


Figure 1. Averages of every 50 temperature readings over time for 1L and 2L PETG bottles in -70°C Storage.

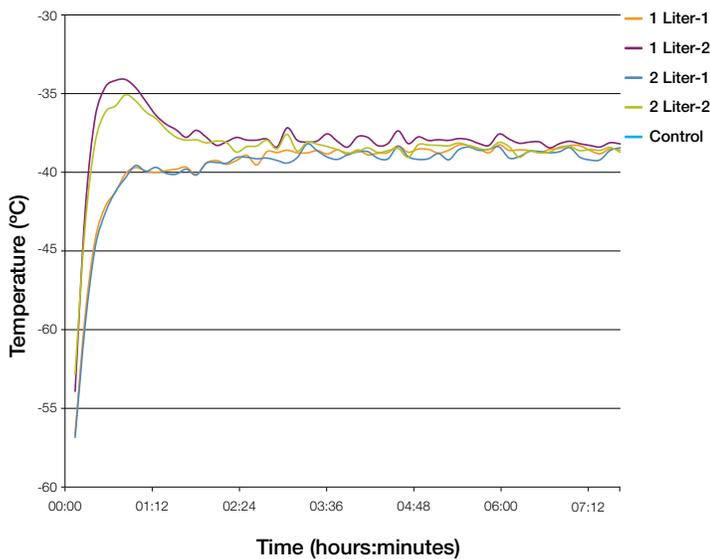


Figure 2. Averages of every 50 temperature readings over time for 1L and 2L PETG bottles transferred from -70°C Storage to -40°C.

### Impact fracture studies

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

Two 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 results in changes in the polymer structure.

### Freeze-thaw back-off torque degradation studies

1L PETG bottles showed 4.69% and 2L bottles showed 7.79% more 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

All 1L PETG bottles passed all pressure leak tests at 10 psi for two minutes and all 2L PETG bottles passed all pressure leak tests at 2 psi for 2 minutes after one freeze-thaw cycle at -40°C and -70°C (Table 3).

Table 1. Impact Fracture Study Results of Bottle Stored at -70°C and Equilibrated to -40°C\*

Test Condition	Sterilization Dose	Sample size (n)	Number of Failures	Failure Rate (%)
1L PETG Bottle	Nominal	30	0	0.00%
	Double	30	2	6.67%
2L PETG Bottle	Nominal	30	0	0.00%

\*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-lbs)	Average Back-off Torque (in-lbs)	Standard Deviation	Torque Degradation (% of Applied)
-40°C	1L PETG Bottle	30	27	3.27	62.10
	2L PETG Bottle	30	38	3.13	57.19
-70°C	1L PETG Bottle	30	27	3.61	66.79
	2L PETG Bottle	29**	38	3.61	64.97

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

Table 3. Pressure Leak Testing Results

Test Condition	Pressure Conditions	Sample size (n)	Number of Failures	Failure Rate (%)	
-40°C	1L PETG Bottle	2 min, 10 psi	30	0	0.00%
	2L PETG Bottle	2 min, 2 psi	30	0	0.00%
-70°C	1L PETG Bottle	2 min, 10 psi	30	0	0.00%
	2L PETG Bottle	2 min, 2 psi	30	0	0.00%

## Discussion

The purpose of this study was to extend the recommended temperature range for existing 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. 1L 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 result in changes in the polymer structure. The risk here is minimal since customers will not encounter product with such a high sterilization dose.

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

While 1L and 2L 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.

## Conclusions

- Both 1L and 2L PETG bottles performed comparably under simulated customer conditions (impact fracture studies).
- 1L and 2L 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 PETG bottles at -70°C is recommended based on the conditions evaluated.

## References

1. Sterigenics, Guidelines for Validation Radiation Sterilization. Retrieved from [http://www.sterigenics.com/services/medical\\_sterilization/contract\\_sterilization/guidelines\\_for\\_validation\\_radiation\\_sterilization.pdf](http://www.sterigenics.com/services/medical_sterilization/contract_sterilization/guidelines_for_validation_radiation_sterilization.pdf) on January 29, 2013.
2. Parks, L.A. Radiation Crosslinking of Polymers. Sterigenics Advanced Applications. 2010. Retrieved from <http://sterigenics.com/>

 Learn more at [thermofisher.com/nalgene](http://thermofisher.com/nalgene)

**thermo**scientific