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Depyrogenation of Vivaspin® Turbo 15 PES in Comparison to Ultrafiltration Devices With a Regenerated Cellulose Membrane

Husna Begum[1,*], Mike Brownleader[2,*], Ben Williams[3]

1. Generon Limited, 11 Whittle Parkway, Progress, Business Park, Slough, SL1 6DQ, United Kingdom
2. Generon Limited, 11 Whittle Parkway, Progress, Business Park, Slough, SL1 6DQ, United Kingdom
3. Sartorius Stedim Lab Limited, Sperry Way, Stonehouse Park, Stonehouse, GL10 3UT, United Kingdom

* Correspondence
E-Mail: adam.green@sartorius.com

Abstract

The presence of endotoxin contamination in biologics and virus based research and discovery can have harmful impacts on target quality, yields and analytical confidence. A common source of contamination is via contact with common laboratory disposables. These disposables are critical to research and discovery workflows and so methods to remove endotoxin contamination prior to use, without effecting disposable functional performance, are increasing critical, along with using disposables with low starting endotoxin concentrations. Here we demonstrate low endotoxin concentrations within the Vivaspin® Turbo 15 PES devices and show further successful depyrogenation using sodium hydroxide. Benchmarking against an alternative device from another supplier was included to highlight the maintained functional integrity of the Vivaspin® devices and their suitability to this application.

Find out more: www.sartorius.com/en/products/lab-filtration-purification/ultrafiltration-devices/centrifugal

Introduction

Endotoxins (or Lipopolysaccharides) are a component of gram-negative bacteria cell wall, an often unwanted impurity in laboratory based research due to their inflammatory and pyrogenic effect on mammalian immune systems.

Here, the background levels of endotoxin from manufacturing are quantified in both Vivaspin® Turbo 15 PES devices and 15 mL ultrafiltration devices from another supplier (Supplier A). Additionally, both types of devices were subjected to treatment of 1 N NaOH, which is commonly used in laboratories as a basic chemical for depyrogenation. A protocol describes the depyrogenation of Vivaspin® Turbo 15 PES for applications where the absence, thus removal of endotoxin is of critical importance.

Method

A) Analysis of typical baseline endotoxin level

1. 2 × Vivaspin® Turbo 15 PES (10 kDa PES membrane) and 2 × 15 mL UF device, Supplier A (10 kDa regenerated cellulose membrane) were selected.
2. Each device was filled with 15 mL HyPure water and left to stand at 20°C for 30 min.
3. Each device was centrifuged at 3,000 *g* for 10 min until approximately 0.5 mL of concentrate remained (approx. 30-fold) in the deadstop pocket.
4. Samples were retrieved from the filtrate reservoir and loaded onto an Endosafe-PTS cartridge for EU/mL quantification.

B) Effect of NaOH treatment on flux and recovery

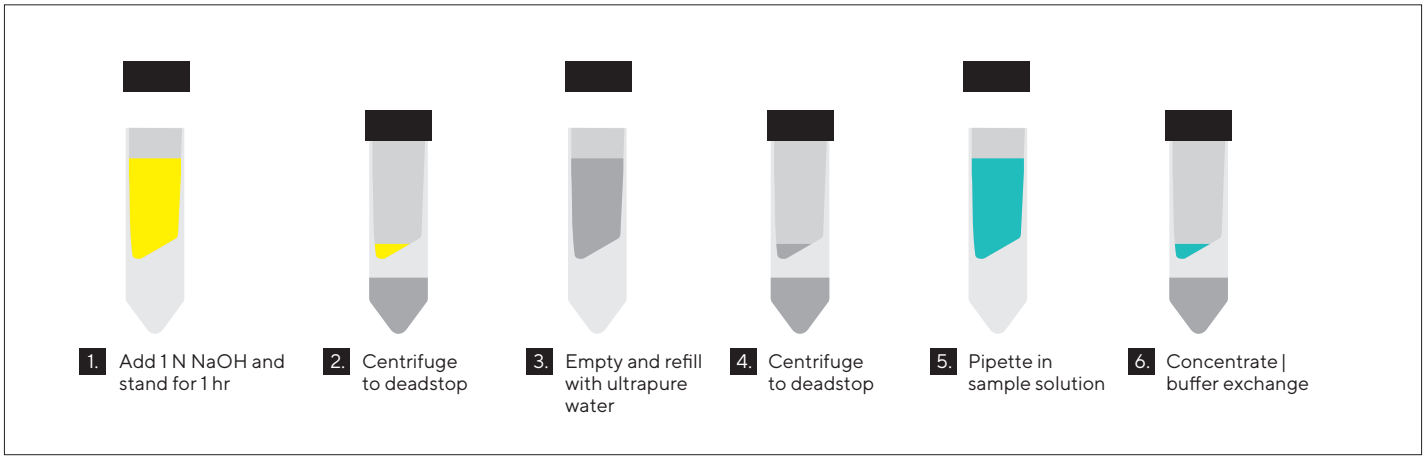
1. 4 × Vivaspin® Turbo 15 PES (10 kDa PES membrane) and 4 × 15 mL UF device, Supplier A (10 kDa regenerated cellulose membrane) were selected.
2. Each device was filled with 15 mL 1 N NaOH and left to stand at 20 °C for 1 hr.
3. Each device was then centrifuged at 3,000 *g* until the device deadstop volume was reached.
4. The devices were emptied, then re-filled with 15 mL HyPure water for the 1st wash cycle.
5. The devices were then centrifuged at 3,000 *g* until the deadstop volume was reached.
6. A 2nd wash cycle was repeated as above.
7. The same devices were then emptied and filled with 15 mL 1.0 mg/mL BSA in saline.
8. All devices were centrifuged at 3,000 *g* until the final concentrate volume was < 0.5 mL.
9. A recovery measurement was then performed on a spectrophotometer.

Results and Discussion

The typical endotoxin levels were an order of magnitude below the guideline maximum threshold of 0.1 EU/mL for intravenous work with a 20 g mouse, showing the inherent cleanliness of the devices in both the Vivaspin® Turbo 15 PES and the 15 mL UF device, Supplier A, even when untreated (table 1).

Upon treatment with 1 N NaOH, the flow rate and protein retention and recovery value in Vivaspin Turbo 15 remained unaffected (table 3). In contrast, the 15 mL UF device with a regenerated cellulose membrane from Supplier A showed a significant reduction in the filtration rate following the use of high pH 1 N NaOH, despite decreasing pH after each wash cycle (table 2).

The total process time following the depyrogenation protocol (described above in method B) was over twice as fast when using the Vivaspin® Turbo 15 PES compared to the 15 mL UF device, Supplier A (table 3).



Schematic depyrogenation process, followed by sample concentration.

Equipment and Test Samples

- Vivaspin® Turbo 15 PES 10 kDa PES (Sartorius, VS15T01)
- 15 mL UF device, Supplier A
- NaOH (Sigma, S0899)
- NaCl (Sigma, S7653)
- HyPure Cell Culture Grade Water, Endotoxin Free (< 0.005 EU/mL) LAL water (HyClone, SH30529.03)
- Albumin from Bovine Serum (Sigma-Aldrich, 1001430867)
- Genova Spectrophotometer (JENWAY, 1282)
- Megafuge 1.0R Centrifuge (Heraeus instruments, 100000494)
- Standard pipettes and tips

Tables and Figures

Table 1: Both the Vivaspin® Turbo 15 PES and 15 mL UF device, Supplier A presented less than 0.01 EU/mL of endotoxin when untreated and tested with a water wash control.

	Vivaspin® Turbo 15 PES		15 mL UF Device, Supplier A	
	1	2	1	2
Final volume	0.54 mL	0.42 mL	0.75 mL	0.52 mL
Endotoxin level	< 0.006 EU/mL	< 0.005 EU/mL	< 0.005 EU/mL	< 0.009 EU/mL

Table 2: Process time taken when devices centrifuged at 3,000 *g*. Depyrogenated Vivaspin® Turbo 15 PES lead to higher recovery of protein after treatment with NaOH. Additionally, the PES membrane remained unaffected by high pH treatment, leading to a faster total processing time by 135 min compared to the time take by the 15 mL UF device, Supplier A.

Device type	Vivaspin® Turbo 15 PES 10 kDa PES	15 mL UF device, Supplier A 10 kDa Regenerated Cellulose
Average time to concentrate BSA 30 × prior to NaOH treatment	15 min	25 min
Average time for NaOH treatment and 2 wash cycles	90 min	225 min
Average time to concentrate protein 30 × post NaOH treatment	15 min	45 min
Final concentrate volume	0.4 mL	0.25 – 0.3 mL
Recovery percentage	97.0%	84.9%
Total process time	105 min	240 min

Table 3: The pH levels of device filtrates were assayed during each wash cycle to demonstrate that even when the pH level was lowered, the negative effect of NaOH on the flow rate of regenerated cellulose was not reversed. The low endotoxin HyPure water and the filtrate from an untreated device presented a baseline pH of 7.55.

	After NaOH treatment	1st wash cycle	2nd wash cycle
pH of filtrate	13.51	11.03	9.32

Conclusion

For applications, in which the absence of endotoxins is essential, we describe a method for fast and reliable depyrogenration of Vivaspin® Turbo 15 PES devices. Additionally, it could be shown that Vivaspin® Turbo 15 PES has superior performance in both flow rate and recovery compared to Supplier A with a regenerated cellulose membrane, following 1 N NaOH soaking treatment for 1 hr.

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Straße 20
37079 Göttingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For further information, visit
www.sartorius.com

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