
Technical Note

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Title: **Sanitization of the Centricon Plus-70 and Centricon Plus-20**

Introduction

Centrifugal concentrator devices are used in a wide variety of applications, including concentration of macromolecules, purification and desalting and buffer exchange by diafiltration. The Centricon[®] Plus-70 centrifugal filter is a disposable, single-use device designed for rapid processing of aqueous biological solutions in volumes ranging from 15 to 70 mL. It is compatible with swinging-bucket centrifuges only. The Centricon Plus-70 device can concentrate most 70 mL solutions down to 350 μ L in just 25 minutes (some solutions may take slightly longer). The information presented in this technical note provides details on the sanitization process used and its effectiveness by comparing colony forming units in the sanitized and non-sanitized devices. The performance of the Centricon Plus-70 was also evaluated by comparing the recovery efficiency of the sanitized device *versus* a non-sanitized control.

Sanitization Procedure

1. Add 70 mL of 70% ethanol to the devices. Incubate the devices for 5 minutes at room temperature.
2. Separately treat the recovery cups with 70% ethanol.
3. Spin the devices at 3500 x g for 25 minutes at 25⁰ C.
4. Rinse the devices and the retentate cups with sterile Milli-Q water until traces of ethanol are removed. Two to three rinses are sufficient.

Notes

- It is critical that the membrane remains wet after sanitization and before usage. Sterile water can be used to keep the membrane wet.
- The procedure described herein is applicable to all Centricon Plus-20 and Centricon Plus-70 devices containing Biomax and Ultracel membranes. The experimental data detailed in this tech note was generated using Centricon Plus-70 devices containing 5K (Biomax PES) and 100K (Ultracel regenerated cellulose) membranes to serve as an example of the utility of the recommended procedure.
- The Centricon Plus-70 is not **autoclavable**.

Efficacy of the Sanitization Procedure

Table.1 shows the effect of 70% ethanol treatment of the Centricon Plus-70 on the number of colony forming units (CFU). The data demonstrate a significant decrease of the number of bacterial colonies observed in the sanitized units *versus* controls. The number of CFUs was determined by adding 70 mL of sterile PBS to sanitized and control Centricon Plus-70 units,

centrifuging at 3500 x g for 25 minutes at 25⁰ C, recovering by a reverse spin, and plating the resulting concentrate on agar plates. The number of colonies observed on each plate was determined after a 48 hour incubation at 37⁰ C.

Table 1. Effect of ethanol sanitization on the sterility of Centricon Plus-70.

Device	Sanitized with 70% Ethanol	Average CFU	% Reduction of Microorganisms
Centricon Plus 70 5k (n=4)	Yes	15.6	87.0
Control (n=2)	No	119.5	
Centricon Plus 70 100k (n=4)	Yes	19.5	86.6
Control (n=2)	No	146	

Performance Evaluation of Sanitized Centricon Plus-70 Devices

The protein recovery of the Centricon Plus-70 was evaluated using two concentrations of test proteins in control (untreated) versus sanitized devices.

Method

Test Devices

Centricon Plus-70.

a- Negative control: Untreated Centricon Plus-70 Devices (5K and 100K)

b- Centricon Plus-70 Devices (5K and 100K membrane) after 70% ethanol sanitization.

Protocol

- Test solutions: Bovine serum albumin (1mg/mL and 0.1mg/mL) for 5K devices and bovine gamma globulin (1 mg/ml and 0.1mg/ml) for 100K devices
- Test solution added to each device.
- Devices placed into the centrifuge with the appropriate rotor.
- Guidelines of the user guide were followed.

Results

Fig 1. Comparison of Protein Recovery in the Sanitized vs. Control Centricon Plus 70 Devices (5k Biomax)

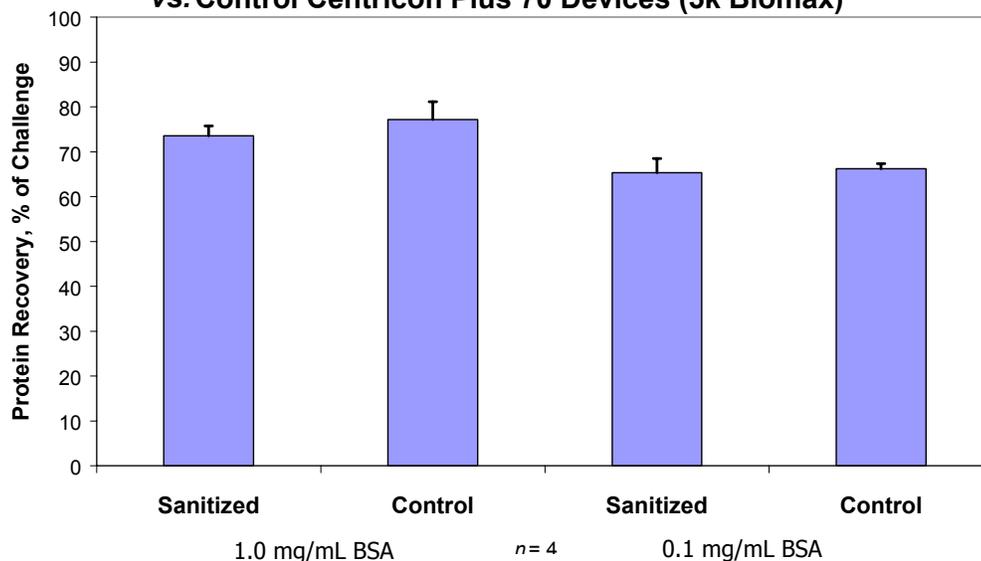
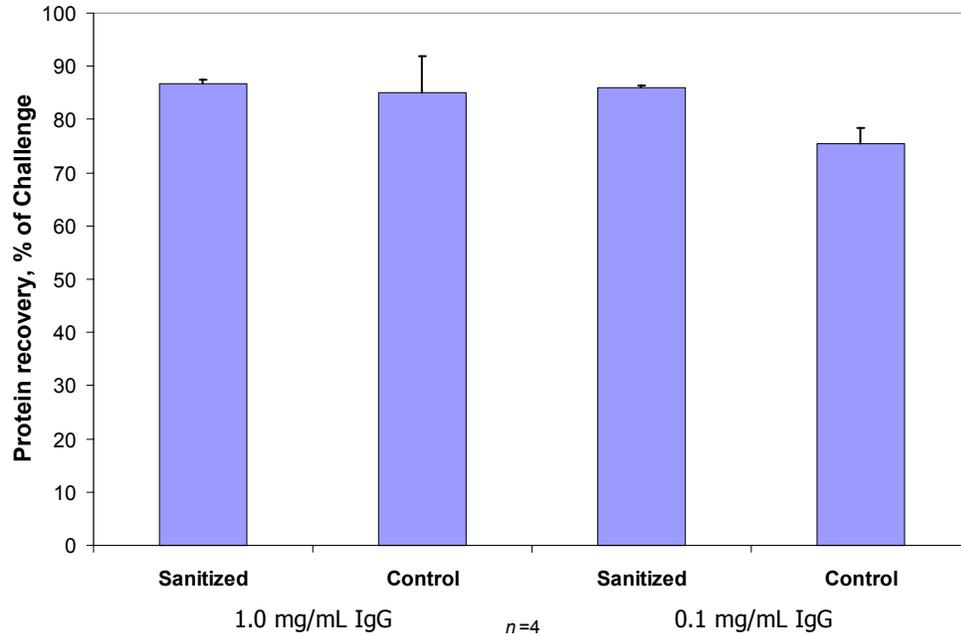


Fig 2. Comparison of Protein Recovery in the Sanitized vs. Control Centricon Plus 70 Devices (100k Ultracel)



Conclusions

The data presented in this tech note clearly demonstrate that the sanitization method described significantly reduced the bacterial load of the Centricon Plus devices. The method also shows that the sanitization procedure does not impact negatively the performance of Centricon Plus. For users of Centricon Plus requiring reduced bacterial loads, sanitization with 70% ethanol is an appropriate option.

