

Gram-Negative Bacterial Lipopolysaccharide Retention by a Positively Charged New-Generation Filter[∇]

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Removing endotoxins is an important target in the pharmaceutical industry and in clinical practice. A filter introduced into an intravenous line prevents microbiological contamination, but to date no filters have retained bacterial endotoxins. In our study, we assayed a new-generation filter which is able to capture endotoxins from solutions.

Endotoxins are lipopolysaccharides (LPS) which constitute the main component of the outer membranes of gram-negative bacteria. Endotoxins at high doses are pathogenic molecules which act as potent activators of the immune system. Monocytes and macrophages, following LPS stimulation, release mediators with powerful biological and pyrogenic activities. Moreover, LPS interact with the vascular endothelium and stimulate the complement and blood coagulation pathways (1, 6).

LPS are formed by three fractions, known as lipid A, core oligosaccharide, and O-antigen (6, 7). Since LPS are partially phosphorylated, the P group confers the net negative charge.

Removing undesirable endotoxins from aqueous solutions is an important aim in the pharmaceutical industry and in clinical practice. Indeed, intravenous (i.v.) therapy is an integral part of modern patient care and is used in the clinical management of the treatment of more than a quarter of hospitalized patients. Unfortunately, i.v. systems also provide a direct route for microorganisms to enter the bloodstream (4). The introduction of a 0.22- μm filter membrane into the i.v. line prevents inadvertent microbial (bacterial, fungal, and yeast) contamination in i.v. fluids from reaching the patient (3, 8). The removal of bacterial endotoxins from liquids is often difficult. The conventional heat sterilization of liquids and filtration with microporous membrane filters, which kill or remove whole bacterial cells, do not eliminate bacterial endotoxins (10, 11). Endotoxins can be eliminated by heating for long periods at elevated temperatures, while the depyrogenation of heat-sensitive biological materials is not feasible. Endotoxins can be removed using ion exchange resins, activated carbon (5), or asbestos-containing filters (9).

Due to the difficulty in removing endotoxins by conventional methods (10), the production of innovative filters able to retain endotoxins is an important goal for the pharmaceutical industry and for medicine. A new filter, known as Speedflow Positive and equipped with a HI-FLO polyethersulfone (PES) 0.2- μm -

pore-size positively charged membrane that electrostatically attracts and/or retains endotoxins, has been produced by GVS SpA (Zola Predosa, Italy) and is referred to hereinafter as filter A. This filter is a modification of a standard filter, known as Speedflow (GVS) and referred to hereinafter as filter C, which guarantees superior flow rate performance and protection against contamination. These new-generation filters (both A and C) are formed by two opposing layers of hydrophilic membranes in a small package; it guarantees mechanical resistance with pump applications, total safety from air embolism, the elimination of large globules in liquids with lipids, and the removal of drug precipitates.

In this study, the two GVS filters A and C were comparatively assayed with two additional filters, B and D, purchased from Pall SpA, Milano, Italy. Filter B is an adult-size 10-cm² positively charged PES membrane with 0.2- μm pores, and filter D is an adult-size 10-cm² standard PES membrane with 0.2- μm pores.

The endotoxin retention assay was carried out with an apparatus which consisted of a test filter, sterile tubing sets, Luer lock outlet connections, a peristaltic pump, and non-pyrogenic glass reservoirs. The depyrogenation cycle was set at 200°C for 4 h.

Pyrotell *Limulus* amoebocyte lysate (LAL) lot no. S05-398 (Associates of Cape Cod [ACC], MA), with a sensitivity of 0.03 endotoxin units (EU)/ml, was used for the gel clot method LAL test. The control standard endotoxin (CSE [lot no. 100; ACC]) was from *Escherichia coli* strain 0113. The accuracy of the gel clot LAL test was established by the range of endotoxin concentrations determined as indicated by the manufacturer (ACC). However, the exact endotoxin concentrations are not known. Nevertheless, the data obtained in our experiments are consistent with the purpose of our work. The reproducibility of the results obtained with the gel clot LAL test was ensured. There was no variability in the results; indeed, we obtained the same data by repeating the experiments 20 times. Results from four representative experiments are shown in Table 1.

Although high endotoxin retention rates (from 1 EU/ml to 10⁶ EU/ml) are demanded for membranes used in environmental microbiology, the removal of endotoxins in the range of 0.03 EU/ml to 1 EU/ml is applied in biomedical fields and in clinical practice, where the concentrations used to assess filters

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TABLE 1. Endotoxin measurements by the LAL test^a

Solution	Filtration time (h)	Amt (EU/ml) of endotoxins in (charged) filter A sample:		Amt (EU/ml) of endotoxins in (charged) filter B sample:		Amt (EU/ml) of endotoxins in (noncharged) filter C sample:		Amt (EU/ml) of endotoxins in (noncharged) filter D sample:	
		1	2	1	2	1	2	1	2
CS	0	1	1	1	1	1	1	1	1
	8	1	0.5	1	1	1	1	1	1
	24	1	0.5	1	1	0.5	1	1	1
	48	2	1	1	1	1	1	1	1
	72	1	1	1	0.5	1	1	1	1
	96	1	1	1	1	0.5	1	1	1
FF	0	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	0.125	0.03
	8	<0.03	<0.03	<0.03	<0.03	0.5	1	0.25	0.5
	24	<0.03	<0.03	<0.03	<0.03	1	0.5	1	1
	48	<0.03	<0.03	<0.03	<0.03	2	8	1	1
	72	0.03	<0.03	<0.03	<0.03	0.5	0.5	2	1
	96	<0.03	<0.03	<0.03	<0.03	0.5	2	1	1
Conclusion ^b		R	R	R	R	NR	NR	NR	NR

^a Results for 2 samples representative of the 20 samples analyzed per filter are shown. Filters A and C were obtained from GVS, and filters B and D were obtained from Pall.

^b R, the filter retained endotoxins; NR, the filter did not retain endotoxins.

are approximately 1 to 2 EU/ml. Indeed, we tested the filters at 1 EU since this is the clinical practice standard for the application of interest (i.v. filtration of clinical solutions). For this reason, our work was focused mostly on the development of a method with replicable results to characterize endotoxin retention at such low levels.

CSE challenge solution (CS) was prepared by dissolving lyophilized CSE in nonpyrogenic water to yield a concentration of 1 EU/ml in 10 liters. To confirm the concentration, a sample of CS was collected and evaluated by the LAL test. The endotoxin retention test was carried out using the following apparatus: a silicon tube edge, attached to the filter, was inserted into the 10-liter reservoir with the 1-EU/ml CSE solution and threaded through an infusion pump; the polyvinyl chloride tube edge terminated in a nonpyrogenic vessel (Fig. 1). The pump was set up to start at 80 ml/h. The first filtrate fluid (FF) was collected at the beginning of the assay, while subsequent

samples of FF and CS which had not passed through the filter were collected after 8, 24, 48, 72, and 96 h of filtration and then tested for endotoxin concentrations by the gel clot method LAL test. All test results were deemed to be valid as the CS yielded the same endotoxin concentration measurement (1 EU/ml) in all the experiments. The endotoxin measurements performed on the filtrates from filters A and B consistently indicated that the amounts of endotoxin which had passed through the filters during the infusion experiment time (96 h) were less than 0.03 EU/ml. On the contrary, the filtrates from filters C and D showed amounts of endotoxin similar to that in the CS (1 EU/ml). From these assay results, we can infer that only filters equipped with a positively charged membrane, filters A and B, were able to retain the endotoxin. Indeed, filters with a standard membrane, filters C and D, did not retain the LPS. This result obtained with purified *E. coli* CSE (ACC) extends the work by Schindler and Dinarello (10). Gerba and

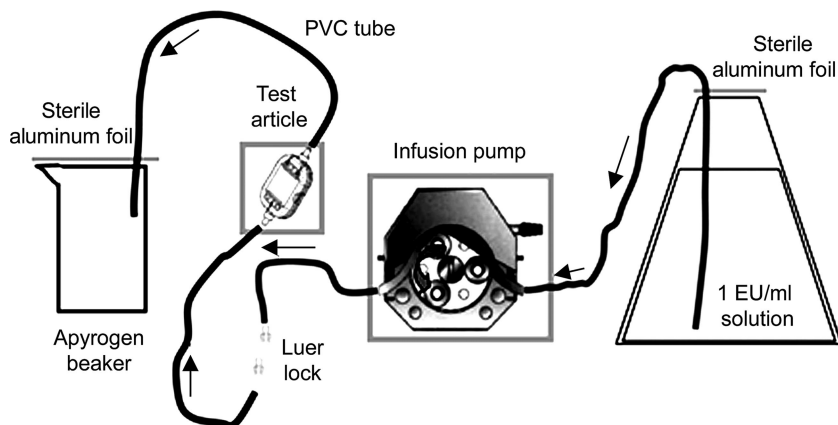


FIG. 1. Apparatus used to carry out the test: an infusion pump was used to test each tubing set and filter, with a 1-EU/ml CSE solution. PVC, polyvinyl chloride.

Hou (2) demonstrated that increasing the net positive charge on filters results in the enhancement of endotoxin retention. Nevertheless, Gerba and Hou (2) obtained good results with depth filters but not with membrane filters.

In this study, we have demonstrated high levels of endotoxin retention using an innovative membrane filter equipped with a positively charged membrane, which electrostatically attracts and/or retains the endotoxins. It is believed that endotoxin retention is mediated by electrostatic interaction forces. Endotoxins easily pass through the 0.2- μm pores of noncharged membrane filters, in which size exclusion is the only retention mechanism. Since endotoxins are negatively charged, the positively charged membrane mounted in our filter may aid the removal of endotoxins, even though these molecules are smaller than the pore size of the filter. The endotoxin retention capabilities of filters equipped with a positively charged membrane indicate that the filters can be safely used for periods of up to 4 days. For this reason, together with endotoxin retention, this filter allows a reduction in therapy cost and treatment time.

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Letter to the Editor

Bacterial Lipopolysaccharide Retention by a Positively Charged Filter

AQ: A

We read with interest the recent paper by Bononi and colleagues (2) concerning the capacity of positively charged membranes to remove endotoxins from solutions intended for intravenous administration. This study raises two issues of concern that should be made known to your readership.

First, the abstract of the article was incorrect in stating that “to date no filters have retained bacterial endotoxins.” Pall Corporation has manufactured positively charged, membrane-based intravenous filter products with a claim for the removal of bacteria and associated endotoxins for the health care community for over two decades. Evidence corroborating this fact is readily available in the peer-reviewed literature (1, 3, 4). We are pleased to see that the results demonstrated by Bononi and colleagues further substantiate the findings in these published works.

Second, there are historical data to suggest that filter performance is influenced by the ionic strength of a salt solution, with filtration efficacy decreasing as ionic strength increases (5). Although the filters evaluated in the referenced studies show performance in the presence of physiologic levels of salt, Bononi and colleagues did not challenge their test filters with saline-based solutions. This leaves open the question of whether or not, in a clinical setting, the new-generation positively charged filters tested will reduce the risk associated with infusing salt-containing intravenous solutions inadvertently contaminated with gram-negative bacteria and associated endotoxins.

We encourage the authors to further characterize this filter, and we look forward to the results of such studies employing more clinically relevant salt-containing intravenous solutions.

We are employees of Pall Medical.

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Authors' Reply

We find reasonable most of the comments and suggestions from Dr. Ortolano and colleagues concerning the article by Bononi et al. (1). In reply to the first comment, we wish to point out that the reduced number of words, no more than 50, published in the abstract of the short-form paper did not allow us to be completely specific in the sentence “A filter introduced into an intravenous line prevents microbiological contamination, but to date no filters have retained bacterial endotoxins,” which should be followed by “with an efficiency of 100%.” Indeed, in our assays, both Pall and GVS positively charged filters were able to retain bacterial lipopolysaccharide. However, the analysis carried out by the *Limulus* amoebocyte lysate test does not allow the challenge of positively charged filters for their absolute efficiency in retaining the bacterial lipopolysaccharide (1). The second comment is correct. Nonetheless, in our experience, GVS positively charged filters performed well in clinical settings. No complaints came to our attention from different hospitals which were GVS customers (our unpublished data). We agree with the final suggestion to carry out additional experiments with “more clinically relevant salt-containing intravenous solutions.” This analysis is feasible, and it will be part of our next study.

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