

BACTERIAL LIPOPOLYSACCARIDES AS COMPLICATION IN INTRAVENOUS THERAPY



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INTRODUCTION

Intravenous (IV) therapy is an integral part of modern patient care and offer many benefits including rapid delivery of drugs and fluids to the patient and haemodynamic monitoring. Unfortunately IV systems also provide a direct route for microorganisms to enter the blood stream. The introduction of a 0.22 µm filter into the IV line offers a simple way of preventing inadvertent microbial contamination of IV fluids from reaching the patient. However this filters are not able to retain endotoxins or lipopolysaccharides (LPS) which are the main component of the outer membrane of Gram-negative bacteria.

Endotoxins are formed by three biologically, chemically, genetically and serologically distinct fractions, named lipid A, core oligosaccharide and O-antigen.

The O-antigen is a heteropolysaccharide built up of a chain of repeating oligosaccharide units (from three to eight monosaccharides each), which are strain specific and determinative for the serological identity of the respective bacterium.

The core oligosaccharide has a conserved structure with an inner sugar-2-keto-3-deoxioctonic acid (KDO)-heptose region and an outer hexose region.

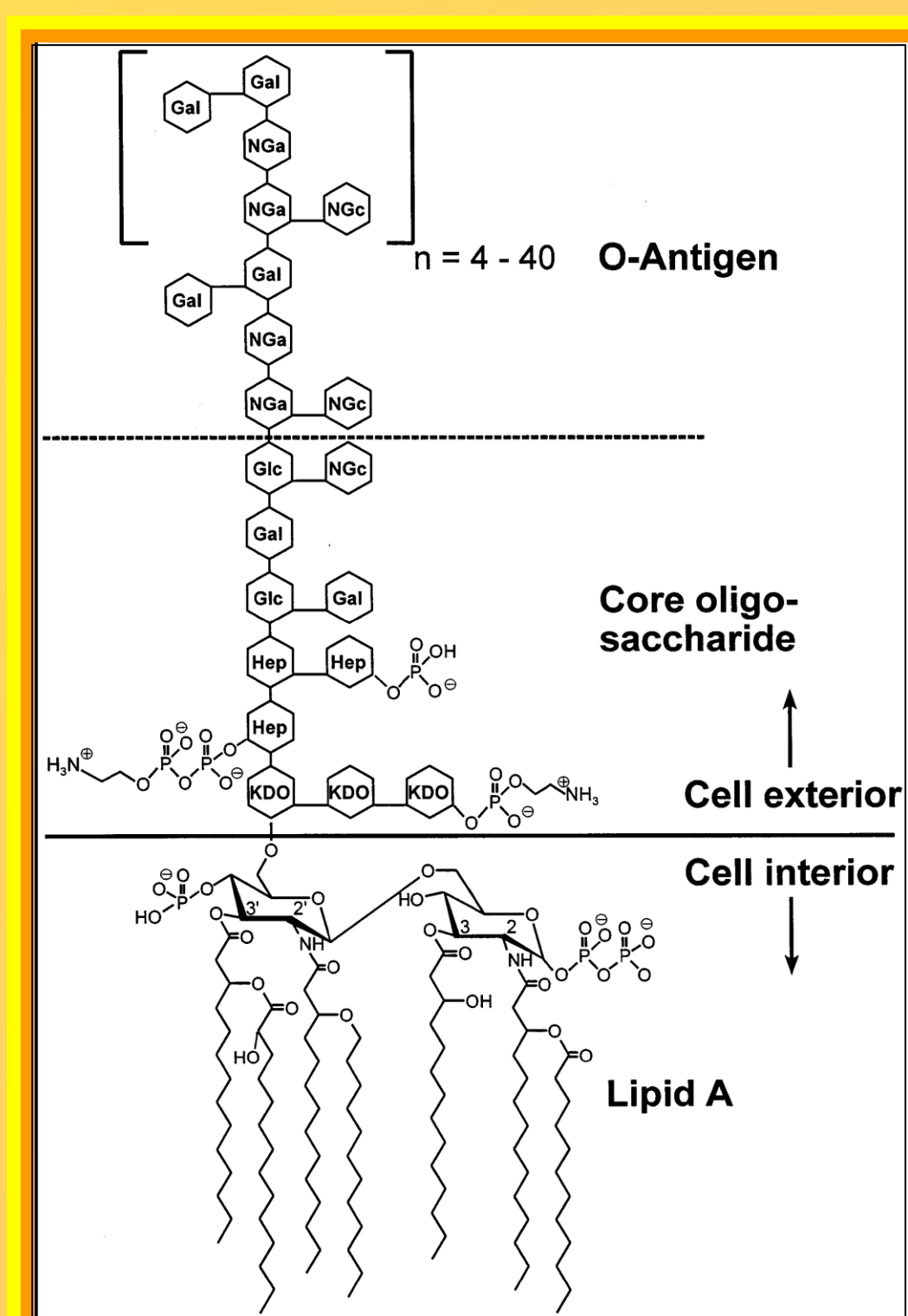
The most conserved part of LPS is the lipid A. The lipid A moiety consists of a diglucosamine backbone attached by ester and amide linkages to long-chain fatty acid of different kind. Lipid A is the endotoxic portion of LPS and its biological activity seems to depend on its molecule peculiar conformation. The lipid A is the hydrophobic portion of endotoxin and it has a neat hexagonal arrangement that results in a very rigid structure in the face of the residue part of the molecule. A are partially phosphorylated thus endotoxin molecules exhibit a net negative charge in common protein solutions (1).

LPS may represent a complication during IV therapy: a parenteral inoculation of high dose of LPS, for example in association with a given drug can caused pyrogenicity, toxicity and inflammation.

Due to the difficulty to remove endotoxins by conventional microfiltration, the creation of innovative filter able to retain endotoxin is a very important target of biotechnological and pharmaceutical industry.

Actually biotechnology and farmaceutic research focused on the development of specific filters able to retani endotoxin (2).

Figura 1:
Lipopolysaccarides (LPS) structure



AIM OF THE STUDY

The aim of the study is to demonstrate whether some filter device retains or not a measured amount of bacterial Control Standard Endotoxin (CSE) when challenged at a defined concentration and flow rate.

RESULTS

In our study we utilized sterile tubing sets with integral filter and Luer outlet connection:

- Filter A: Speedflow Adult Filter 10sqcm PES membrane 0.2 positively charged manufactured by GVS
- Filter B: Adult Filter 10sqcm PES membrane 0.2 positively charged manufactured by competitor A
- Filter C: Speedflow Adult Filter 10sqcm PES membrane 0.2 standard manufactured by competitor A
- Filter D: Speedflow Adult Filter 10sqcm PES membrane 0.2 standard manufactured by GVS

We created a membrane positively charged because it would be able to retain endotoxin that have a negative charge due to the phosphorilations of Lipide A.

Preliminary experiments demonstrated there was no adventitious binding of CSE to containers utilized. All tests were deemed valid because the challenge solution yields a similar concentration value (1 EU/ml) from the beginning to the end of the test procedure.

The endotoxin measurements performed on the filtrates of samples A and B consistently indicate that the amount of endotoxin, passed through the filter during the time of the experiment (96 hours) of infusion, is less than 0,03 EU/ml.

On the contrary the filtrates from samples C and D show an amount of endotoxin similar to the challenge solution (1 EU/ml). This result means that endotoxin passed through these two last filters during all the time of the experiment (Table 1).

CONCLUSIONS

Our data indicate that both articles A and B, filters with a charged membrane, retain endotoxins and that articles C and D, with not charged membrane allow endotoxins to pass in the filtrate fluid.

ACKNOWLEDGEMENTS

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Figure 2A



Figure 2: Apparatus used to carry out the test: A infusion pump was used to challenge every tubing set, and so every filter, with the 1 EU/ml CSE solution, as we illustrated in figure 2A and as we draw in figure 2B. Samples of filtrate fluid (FF) and challenge solution (CS) were collected after 0, 8, 24, 48, 72 and 96 hours of flow through the filter. The samples were tested for endotoxin concentration with LAL test gel-clot method.

Figure 2B

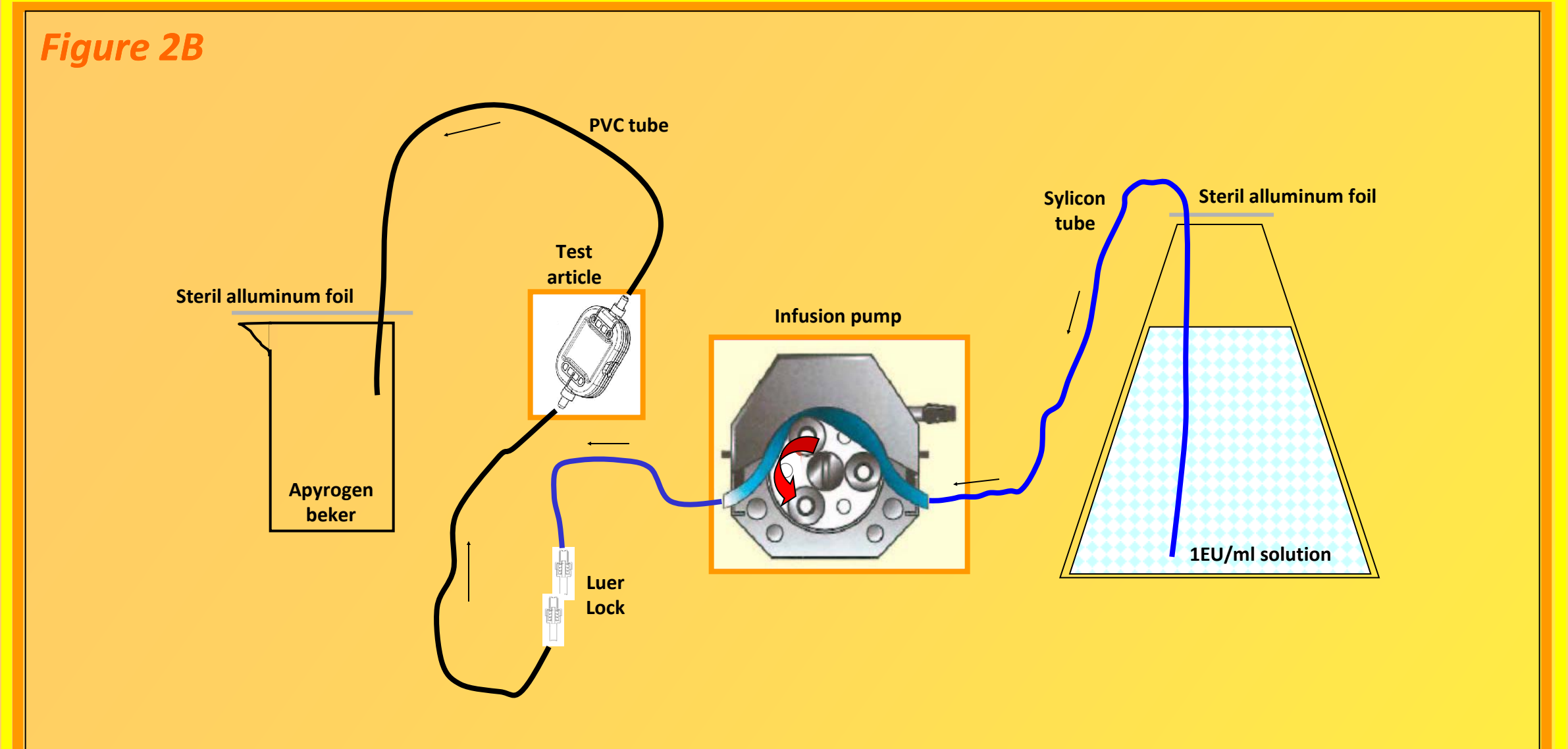


Table 1: Endotoxin measurements (EU/ml) by LAL test

Solution	Filter hours	A	B	C	D
		Challenge Solution	0	1	1
	8	1	1	1	1
	24	1	1	1	0,5
	48	2	1	1	1
	72	1	1	1	1
	96	1	1	1	0,5
Filtrate fluid	0	<0,03	<0,03	0,125	<0,03
	8	<0,03	<0,03	0'25	0,5
	24	<0,03	<0,03	1	1
	48	<0,03	<0,03	1	2
	72	0,03	<0,03	2	0,5
	96	<0,03	<0,03	1	0,5

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