

Removal of Endotoxin using Point-of-Use Filters

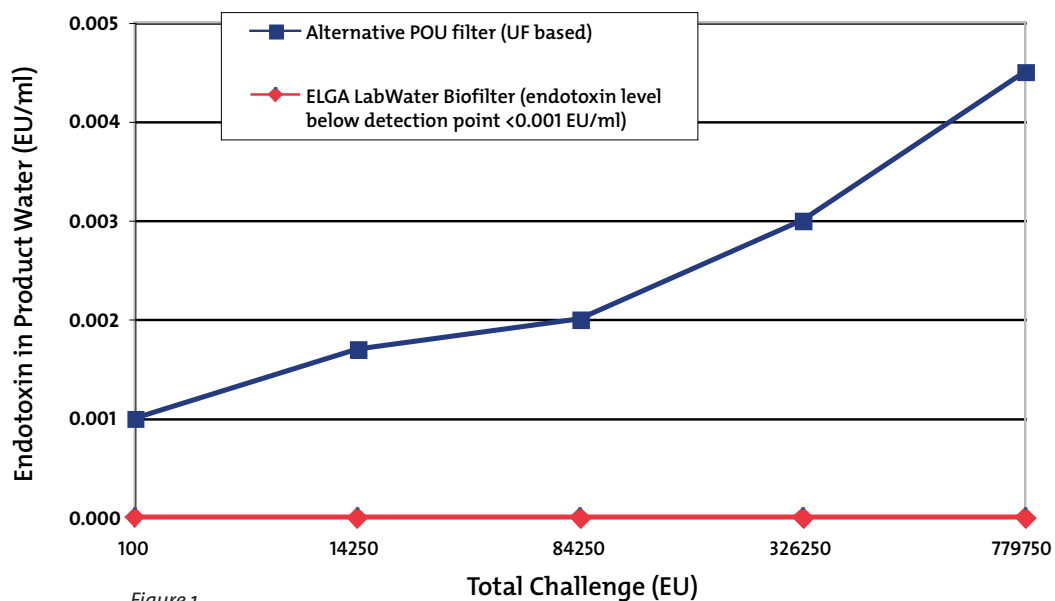
Endotoxins are lipopolysaccharides (LPS) shed from the outer membrane of gram-negative bacteria. They are released when the bacterial cell dies. Endotoxins interact with cells causing a wide range of detrimental effects (Ref 1. Dawson and Ref 2. Nagano). Other applications such as in-vitro fertilization (Ref 3. Dumoulin) and cell culture (Ref 4. Stacey) are very vulnerable. Reliable experimentation involving cell division, electrophoresis and other biochemical processes all benefit from the removal of endotoxins.

Endotoxins are negatively charged at pH >2 and can be efficiently removed by positively charged filters such as the ELGA LabWater Biofilter. Charged filters provide minimal obstruction to water flow and are the preferred option for a point-of-use application when they are used at the final stage of a series of purification techniques.



The physical barrier used in an ultrafilter (UF) can restrict the flow unless they have a large surface area (as in in-line applications) or, where large size is not acceptable, such as in point-of-use (POU) devices, some compromise is made on performance. These ultrafilters are not absolute and allow the passage of some larger molecules. Rather than solely relying on a single point-of-use ultrafiltration, a total system approach is more effective. It has the benefit of a combination of technologies such as ion exchange, UV, UF and chemical sanitization.

ELGA Biofilter and an Alternative Point-of-Use Filter (UF based) Endotoxin Challenge



Both point-of-use filters were challenged by continuously adding high levels of endotoxin to the water fed to the filters. The concentration of endotoxin was then measured in the product water using the limulus amoebocyte lysate test (kinetic turbidimetric assay type).

Most endotoxin challenges rely on purified LPS. The research team at ELGA LabWater produced its own LPS from bacteria already present in purified water. This was to imitate a realistic challenge environment. Initially the bacteria were isolated from purified water. The microorganisms were inoculated into peptone water and then incubated at 27°C. The product was repeatedly autoclaved and filtered using a 0.45µm filter membrane which resulted in concentrated endotoxin.

Each challenge lasted 5 minutes giving the total challenge values in figure 1, even at over 90 EU/ml and a total loading of nearly 800,000 EU, no endotoxin (<0.001 EU/ml) could be detected in the product water from the Biofilter. This is equivalent to >5 log reduction.

As well as removing any endotoxin in the product water from the unit, such point-of-use devices must not contaminate the water. The rapid resistivity and TOC rinse up of new filters when they are first used is of great benefit to the user and a good indication of an on-going minimal contribution of contamination to the product water. This is critical, as the water purity can not be monitored after the filter. The rapid rinse up of the Biofilter for TOC is shown below. The TOC rinse up is more efficient than an alternative point-of-use filter based on ultrafiltration.

TOC Rinse-up of Biofilter & Alternative POU Filter (UF based)

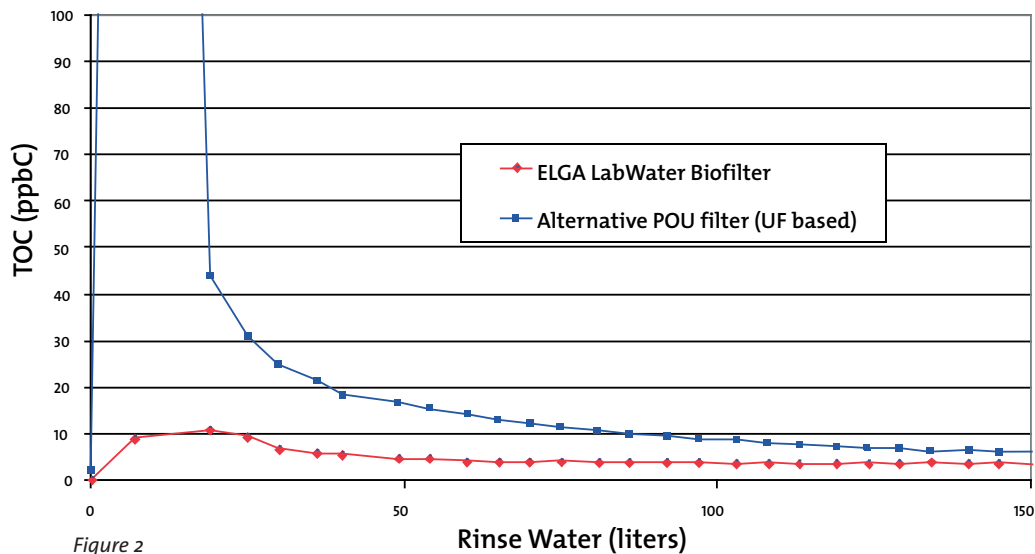


Figure 2

Elimination of endotoxin contamination requires a combination of effective bacterial control via UV, chemical sanitization and optimal in line POU filtration. Positively charged filters provide the ideal means to remove final traces of endotoxin from ultra-pure water systems. The Biofilter is highly recommended for use as a positively charged filter and provides negligible contribution to contamination.

References

- Ref 1: Dawson ME (1998) LAL update. Associates of Cape Cod; Vol. 16: 1-4
- Ref 2: Nagano M, Takahashi Y, Katagiri S (1999) J. Reprod. Dev.; 45: 239-242
- Ref 3: Dumoulin JC, Menheere PP, Evers JL (1991) Human Reproduction; 6: 730-734
- Ref 4: Stacey G (2007) in Medicines from Animal Cell Culture. Stacey G, Davis J. John Wiley & Sons, Chichester, Chapter 31

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