Removal of Endotoxin, RNase, DNase and Bacteria using the PURELAB® Chorus 1 fitted with a Biofilter

Performance of ELGA Biofilter fi tted to a PURELAB Chorus 1

Specifications

- Endotoxin <0.001 EU/ml
- RNase <0.002 ng/ml
- DNase <20 pg/ml (<0.02 pg/µl)
- Bacteria <10 CFU/100ml (<0.1 CFU/ml)

The PURELAB Chorus 1 provides ultrapure water directly from a pretreated water supply. When fitted with an ELGA LabWater Biofilter it provides water, which is effectively free from biologically active impurities.

This then ensures that the water is suitable for use with biochemical applications such as cell culture.

Endotoxins

Endotoxins are lipopolysaccharides (LPS) shed from the outer membrane of viable gram-negative bacteria. They are released when the bacterial cell dies.

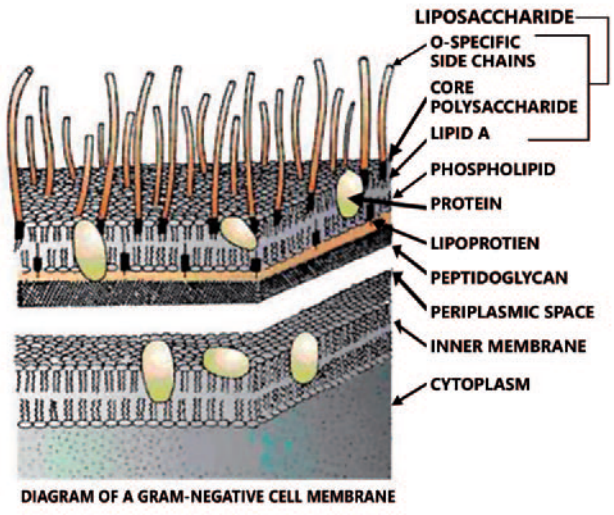

DIAGRAM OF A GRAM-NEGATIVE CELL MEMBRANE

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 PURELAB Chorus 1 for a point-of-use application when they are used at the final stage of a series of purification techniques.

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TECHNOLOGY NOTE 30

Endotoxin Challenge

The Biofilter was challenged by continuously adding high levels of endotoxin to the water fed to the positively charged filter. 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 designed 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 below. 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.

Challenge (EU/ml)	0.02	2.83	14.00	48.40	90.70
Challenge Total (EU)	100	14250	84250	326250	779750
Post Biofilter (EU/ml)	<0.001	<0.001	<0.001	<0.001	<0.001
Log Reduction	>1.3	>3.5	>4.1	>4.7	>5.0

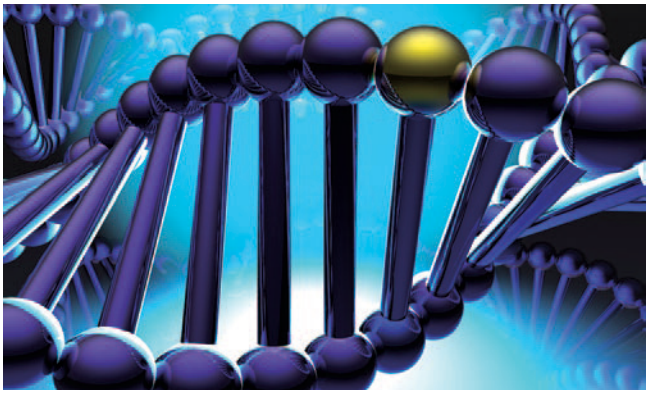
Table 1

In practise, the level of endotoxin in the feed water to the filter will be very low (<0.1 EU/ml). To imitate these conditions more closely, the filter was then tested over a prolonged period with a nominal feed of 1 EU/ml. No endotoxin (<0.001 EU/ml) could be detected after the passage of 800 liters of water containing 1 EU/ml.

DNase and RNase

Concerns have been raised about the possible presence of other biologically active species, such as RNase and DNase in purified water.

The presence of these species in purified water could cause serious interference. However they are removed by ion-exchange media and exposure to oxidizing UV light, and have not been found in the water from well-designed systems that have been properly sanitized and maintained.



In-house enhanced versions of Ambion® Alert test procedures, based on the use of cleavable fluorescent labelled RNase or DNase substrates were used for detection. Test results show RNase detection levels as low as <0.002 ng/ml and DNase levels at <20 pg/ml. Even with these high levels of sensitivity, the water produced by the PURELAB Chorus 1 fitted with a Biofilter was effectively free from RNase and DNase (RNase <0.002 ng/ml, DNase <0.02 ng/ml). At these levels, purified water from the PURELAB Chorus 1 fitted with a Biofilter can be used instead of water subjected to Diethylpyrocarbonate (DEPC) treatment (subject to regular sanitization and maintained according to ELGA LabWater recommendations).

To further test the system, the PURELAB Chorus 1's storage reservoir was filled with a challenge of 1mg/l of RNase A and DNase i.e. a factor of 500,000 or more above the detection limit.
The product water was effectively free from RNase and DNase (RNase <0.002 ng/ml, DNase <0.02 ng/ml).

TOC and Resistivity Rinse Up

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 a considerable convenience and a good indication of an on-going very low 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 both TOC and resistivity is shown in Figure 1.

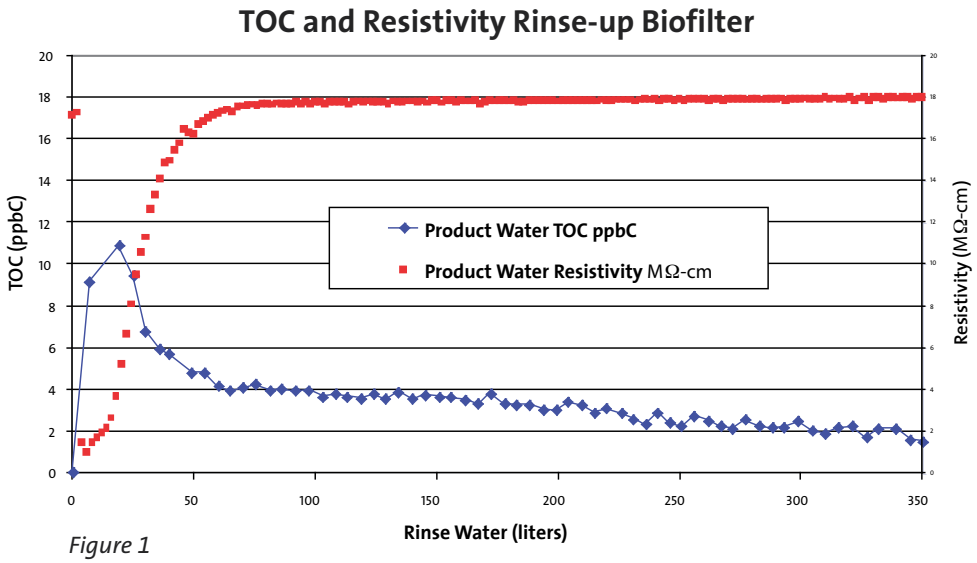


Figure 1

Bacteria Challenge

Within the PURELAB Chorus 1, the bacterial levels are maintained at very low levels by initial reverse osmosis filtration and by subsequent recirculation through a UV chamber providing exposure to intense UV light at 254 and 185 nm. The formation of biofilm is restricted by regular sanitization. Final traces of bacteria are removed by the Biofilter, which provides 0.2µm bacterial filtration. When challenged with a feed solution containing 1 x 10^7 CFU/ml, the Biofilter removed bacteria equivalent to a Log reduction of >8.

Typical values (Total Viable Count - TVC) from an PURELAB Chorus 1 fitted with a Biofilter over a 6 month period are highlighted in Figure 2.

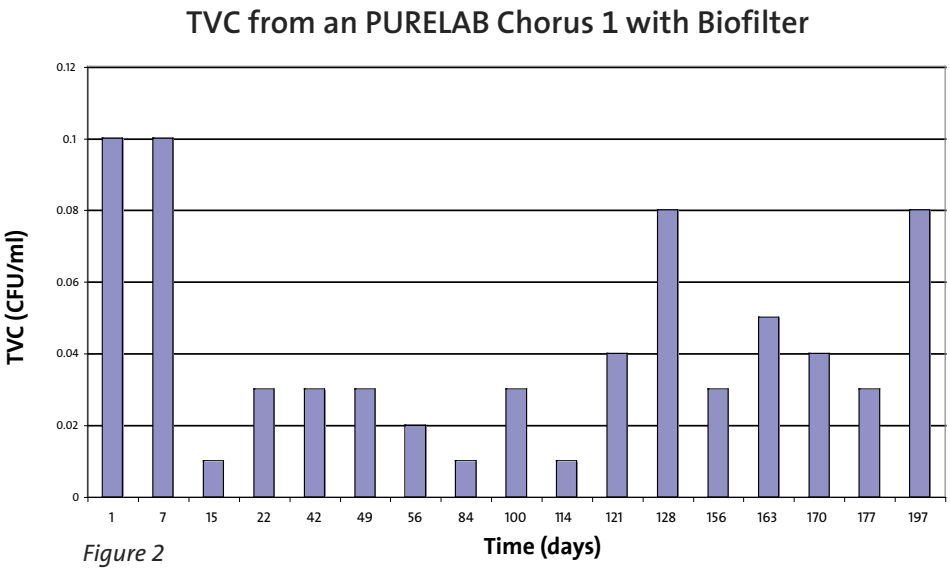


Figure 2

Summary

The Biofilter when fitted to the PURELAB Chorus 1 is very effective for producing water which is free from biologically active impurities. This makes it suitable for use with applications which require endotoxin free ultrapure water, bacteria free water, and nuclease free ultrapure water.

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 Whiley & Sons, Chichester, Chapter 31

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