

Volume 16  
October 2012

SCREENING  
TRENDS IN DRUG DISCOVERY

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G.I.T.

LABORATORY  
JOURNAL

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10  $\mu\text{m}$ Microscopic picture of  
PER.C6 cells in suspension  
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# Water is Life

## Ultra Pure Water in Cell Cultivation



**Water is a major component of all cell culture media and is therefore needed to prepare media, buffers, and additives, and as well as to serve many ancillary functions, such as heating, cooling, cleaning and rinsing. Thus, water quality plays an important role in the outcome of cell culture experiments.**

Contaminants in water used for cell cultures can occur in many forms, such as bacteria, yeasts or molds. These contaminants are usually visible to the eye or by optical microscopy. However, contamination from chemicals or other biological agents may also affect growth, morphology or behavior of cultured cells, yet be undetectable to the unaided eye. Water used in cell cultures must therefore be free of microorganisms and, in particular, of endotoxins, inorganic ions (heavy metals such as lead, zinc, etc.), and organic compounds (humic acids, tannins, pesticides, etc.). For more detailed information, please refer to the reference literature [1, 2]. Examples of typical impurities in mains water (tap water) and target values for cell culture work are shown in table 1.

The objective of the present test series was to evaluate whether ultra pure water produced by Arium pro UF can be readily used for cell culture applications without entailing any problems. In this study, we cultivated PER.C6 EpCAM cells in ready-made CDM4PERMab (Hyclone) media employed as controls, as well as in CDM4PERMab (Hyclone) powder media prepared using ultrapure water obtained with Arium pro UF (UF water) and RO water, respectively, for test purposes. Data for RO water in this Application Note were obtained

**Fig. 1: Arium pro UF ultrapure water system**  
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using the predecessor model (Arium RO) of the current Arium advance system. The results of each culture were then used to assess whether the water produced by Arium pro UF is suitable for use in the cultivation of PER.C6 EpCAM cells.

The PER.C6 cell line derived from human retinoblast cells described and employed in our test

Parameter	Mains water	Water for cell culture	% reduction
Conductivity ( $\mu\text{S} / \text{cm}$ )	50 to 900	0.2	99.95
Calcium (mg / l)	20 to 150	< 0.01	> 99.99
Sodium (mg / l)	20 to 150	< 0.01	> 99.99
Iron (mg / l)	0.01 to 0.1	< 0.001	> 98
Bicarbonate (mg / l)	30 to 300	< 0.01	> 99.99
Chloride (mg / l)	10 to 150	< 0.01	> 99.99
Sulphate (mg / l)	1 to 100	< 0.01	> 99.98
TOC (mg / l)	0.2 to 5	0.1	96
Free chlorine (mg / l)	0.1 to 0.5	< 0.01	> 97
Bacteria (CFU / 100 ml)	100 to 1000	< 10	> 98
Endotoxin (IU / ml)	1 to 10	< 0.1	> 98
Turbidity	0.1 to 2	< 0.01	> 99

**Table 1: Typical mains water impurities and target values for cell culture work [2].**

series is also used today for the expression of recombinant proteins and monoclonal antibodies (Mab production) and for the manufacture of therapeutic proteins and monoclonal antibodies.

### Ultrapure Water System

The system (fig. 1) is designed to produce ultrapure water from pre-treated water sources by removing trace levels of residual contaminants. Production of ultrapure water requires continuous recirculation and constant flow. This is carried out by a pump system that controls the pressure. The system measures the conductivity of the water at both the water feed inlet and the water outlet (downstream product).

The system works with two different cartridge kits. These cartridges are filled with a special active carbon adsorber and a special mixed-bed exchange resin designed to deliver high-purity water with low extractables. A final microfilter at the outlet is usually installed to remove any particles or bacteria from the ultrapure water as it is dispensed. The general process described for water purification is depicted in figure 2.

For the tests mentioned below the feed water for the Arium pro UF system (predecessor model with the same technical design as that of the current Arium pro UF device shown in fig. 1) was prefiltered by an Arium RO reverse osmosis system. This configuration is in accordance with Whitehead [2] for the water purification treatment for small scale cell cultivation in laboratories. This pretreatment system is not further specified in this Application Note.

### Materials and Methods

PER.C6 EpCAM cells (eye catcher) were cultivated in T-75 flasks, with vented caps (Nunc) for gas exchange, in duplicates (12 ml media in each flask) for 10 passages and in 125 ml spinner flasks (Wheaton, VWR) with 50 ml of media in duplicates. The PER.C6 EpCAM cell line was cultivated in CDM4PERMab ready-made media (Hyclone) and in CDM4PERMab powder media (Hyclone). The CDM4PERMab powder media was reconstituted with either UF water or with RO water, along with 4 mM L-glutamine (Lonza), sodium bicarbonate (3.2 g / l, Merck) and pluronic acid F-68 (0.5 g / l, Sigma), and filtered through a final 0.2  $\mu\text{m}$  sterilizing-grade filter using 1000 ml disposable filtration units (Sartolab, Sartorius) under aseptic conditions.

The cells were seeded at a seeding density of  $0.3 \times 10^6$  cells / ml in T-75 flasks and  $0.7 \times 10^6$  cells / ml in spinner flasks. The T-flasks and spinner flasks were incubated in a CO<sub>2</sub> incubator (Forma direct heat CO<sub>2</sub> incubator, Model 3-11 Thermo Scientific) at 37°C, 5% CO<sub>2</sub> and 85% humidity. In the CO<sub>2</sub> incubator, the spinner flasks were incubated on a magnetic stirrer (VWR) at 80 rpm with the side arm of each spinner flask loosely capped to facilitate gas exchange inside. Samples were taken every day except for week-

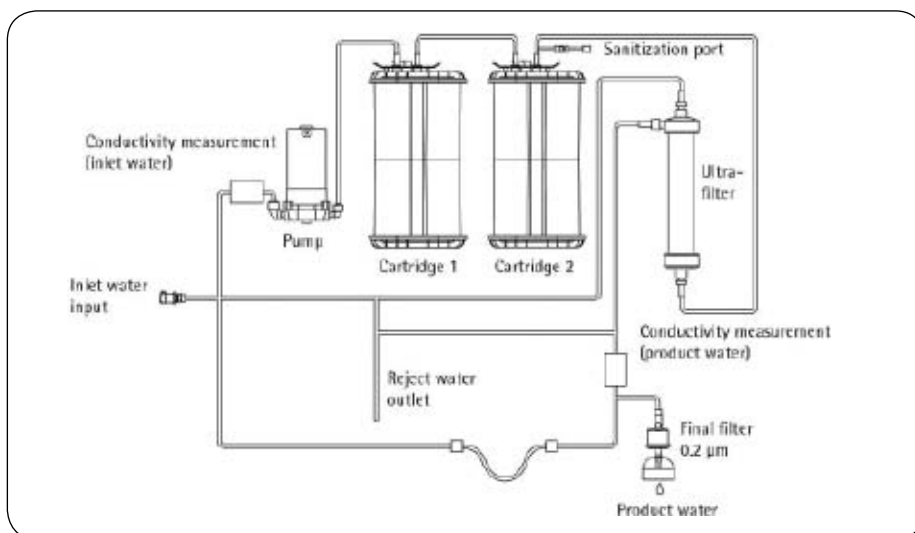


Fig. 2: Schematic drawing of the Arium pro UF ultrapure water system.

ends (days 4 and 5) from the spinner flasks and every third day from the T-flasks to determine the viable cell density. The viable cell density was measured according to the Trypan Blue exclusion method using a hemacytometer (Vasa Scientific). Comprehensive information about the basic techniques of cell cultivation is given in the reference literature [3].

### Results and Discussion

The average cell density yield in the control T-flasks (cells cultivated in ready-made media as controls) was  $1.52 \times 10^6$  cells / ml and the average viability for these controls was 95.23% (fig. 3a). For cells in T-flasks cultivated in media reconstituted with UF water, the average cell density was  $1.73 \times 10^6$  cells / ml, and an average viability of 95.7% was obtained. In comparison with these results, an average cell density yield of  $1.68 \times 10^6$  cells / ml and a viability of 95.59% (fig. 3a) were obtained in the cells cultivated in media reconstituted in RO water.

In another experiment, the PER.C6 EpCAM cell lines were cultivated in spinner flasks with ready-made media (control), media reconstituted using UF water and media reconstituted using RO water (fig. 3b). The maximum cell density obtained in control spinner flasks was  $5.42 \times 10^6$  cells / ml with an 88.47% viability,  $6.24 \times 10^6$  cells / ml with an 88.55% viability in UF water spinner flasks and  $4.60 \times 10^6$  cells / ml with an 89.85% viability in RO water spinner flasks on day 6 of cultivation (fig. 3b). Microscopic examination of the cells in the RO spinner flasks indicated that cells grown in media reconstituted with RO water look unhealthy compared with cells grown in the ready-made media and in media reconstituted with UF water. The viability of the cells grown in spinner flasks with media reconstituted in RO water decreased more rapidly compared with those cultivated in the ready-made media and media reconstituted with UF water. This decrease was not observed when the cells were cultivated in T-flasks. The rapid decline

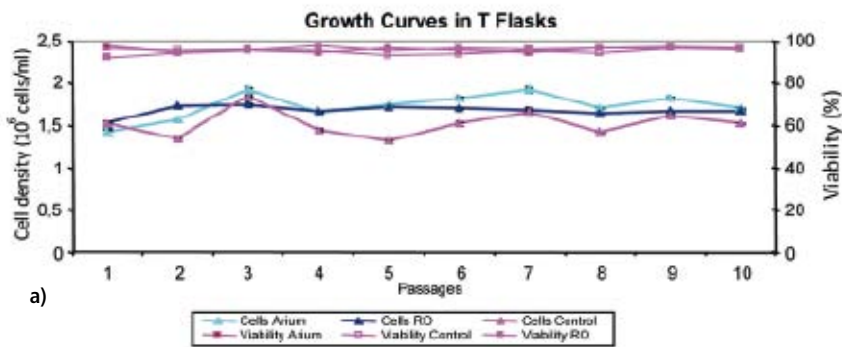
in viability inside the spinner flasks can be attributed to the presence of endotoxins and inorganic salts present in RO water which affect the growth and viability of the cells. However, these adverse effects of endotoxins or inorganic salts were not observed in static cultures (small-scale cultivation), such as in the T-flasks, because in the latter case, growth is limited by the O<sub>2</sub> concentration in the medium and not by the endotoxin or inorganic salt concentration (no typical growth curve can be observed in the T-flasks compared with the cultures in the spinner flasks).

The actual effects can be observed only in spinner flask cultivation where higher cell densities occur and O<sub>2</sub> is not a limiting factor. In spinner flasks in which high cell densities can be achieved, the effect of higher concentrations of endotoxins and inorganic salts in media reconstituted with RO water result in a reduced growth rate (lower cell density and lower viability) compared to values obtained for the controls (ready-to-use media) or for the samples in medium reconstituted with UF water. These results are confirmed by the antibody production (Mab) experiments. Mab production in spinner flasks (fig. 4) of cells cultivated in media reconstituted with UF water was 0.84 mg / ml (example day 8) and is thus higher than the values obtained for Mab production with the manufacturer's ready-made media as controls (0.71 mg / ml) or in the samples reconstituted with RO water (0.42 mg / ml).

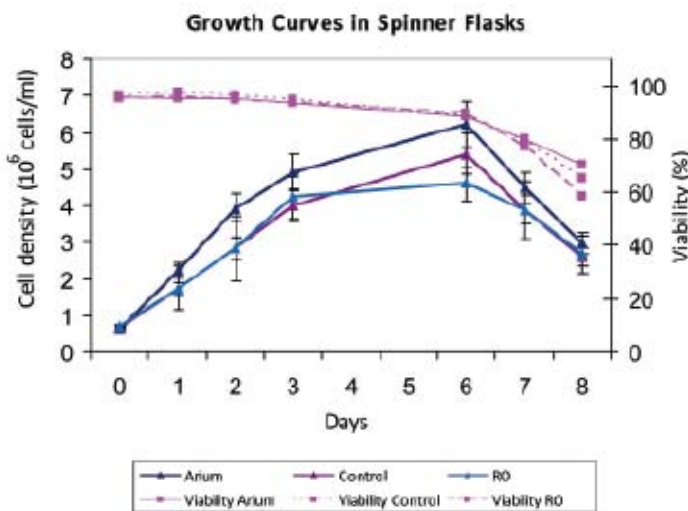
The productivity of cells, i.e. Mab production in T-flasks was not measured because the amounts of antibodies were too low and a reliable comparison of such low values was not statistically relevant.

### Conclusions

The results above clearly demonstrate that dehydrated media (CDM4PERMab media) that are reconstituted with UF water are suitable for use in cultivation of PER.C6 EpCAM cell lines instead of commercially available ready-to-use media. The growth characteristics of the PER.C6 EpCAM cell lines cultivated in media reconstituted



a)



b)

Fig. 3a: Growth curves of PER.C6 EpCAM cell lines in T-flasks

Fig. 3b: Growth curves of PER.C6 EpCAM cell lines in spinner flasks

### Antibody Production in Spinner Flasks

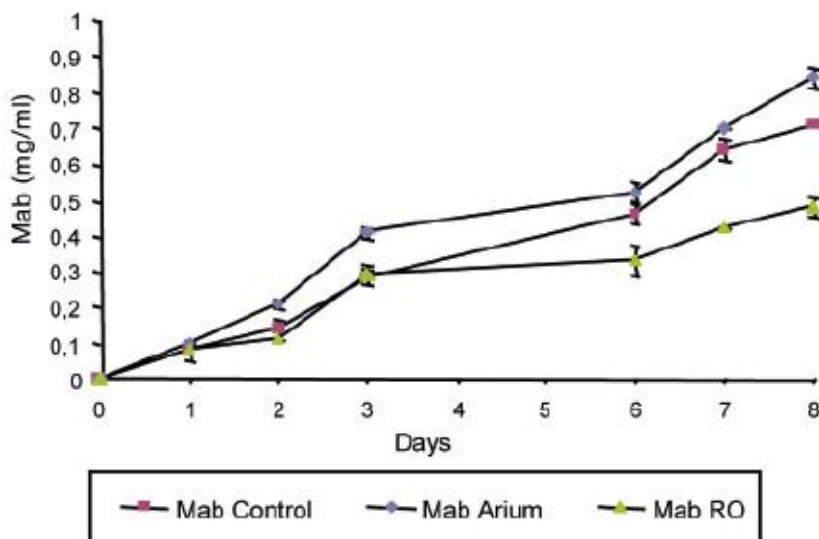


Fig. 4: Antibody production in cells cultivated in media reconstituted with UF water (Mab Arium), ready-made media (Mab Control) and RO water (Mab RO) in spinner flasks

with UF water are similar to those of the PER.C6 EpCAM cell lines cultivated in ready-to-use CD-M4PERMab media used as controls.

Moreover, we observed enhanced growth in cell line samples cultivated in media reconstituted with UF water compared with the samples cultured in media reconstituted with RO water when the experiments were carried out in spinner flasks. In this case higher cell densities normally occur and O<sub>2</sub> is not a limiting factor. We therefore conclude that the higher concentration of endotoxins or inorganic salts in RO water caused this decrease in growth.

These results were confirmed and reflected by the Mab production in the PER.C6 EpCAM cell line cultivated in spinner flasks. Mab production from PER.C6 cell lines showed the highest values in the samples reconstituted with Arium pro UF ultrapure water, followed by those of the controls (ready-to-use media). These values were unlike those in the RO water samples, where Mab production decreased.

Hence, we conclude that water from the Arium pro UF system is excellently suited for PER.C6 EpCAM cell cultivation because this water system minimizes the content of impurities, such as inorganic ions, organic compounds and, in particular it reduces endotoxins to exceptionally low levels, as was recently confirmed in newer experiments [4].

### Reference Literature

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- [3] Freshney I. R.: Culture of Animal Cells – A Manual of Basic Technique and Specialized Applications- 6th edition, John Wiley & Sons, Inc., USA (2010)
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### Acknowledgement

We would like to acknowledge Dr. Alexander Tappe and Dr. Yvonne Martin of Sartorius-Stedim Biotech GmbH, Goettingen, Germany for providing the photograph of the PER.C6 cell line and further technical discussions.

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