

# UltraPure Water Application Culture of Nerve Cells

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**Key Words** : \* *Nerve cells, cell culture, ultrapure water, pyrogen removal*

**Summary** : \* This paper describes the results of various nerve-cell cultures using water with varying levels of purity. The necessity for pyrogen and organic-free ultrapure water in such experiments is shown. A system allowing direct production of high purity water is proposed.

## **Introduction**

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The requirement of pure water for media preparation and washing materials for mammalian cell culture is well known. The presence of metal ions and organics can be very harmful to cell culture and can prevent healthy cell growth. There have been various reports regarding the influence of fever-generating compounds (pyrogens) in water<sup>i, ii</sup>. The great influence of water quality on nerve cell survival and growth in serum-free media or media containing a low concentration of blood serum is well known.

However, many instructions remain unclear in the procedures for “pure water” preparation, and many researchers use deionized water that has been distilled two to three times guided only on previous experience. Water purification via double or triple distillation requires a large amount of time and is subject to contamination by organics, pyrogens, and other materials from the environment and storage containers. The Milli-Q<sup>®</sup> SP UF system, introduced here, uses ultrafiltration by means of a hollow fiber ultrafiltration cartridge with clearly defined separation characteristics.

This device consistently and efficiently removes pyrogens, proteins, and other materials, to produce ultrapure water that can be used directly at point of use unlike traditional “pure water” produced by other methods.

Direct production and use ensures a high level of water quality for primary nerve-cell culture.

## **Experiment**

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### **Cells**

Cells were separated from the brain of Weister rats 16 to 17 days into gestation. Cells were plated as  $1-2 \times 10^5$  cells/well (24 wells) on a microplate coated with poly-L lysine. After a 3-day culture, cells were fixed with 4% gluteraldehyde and stained with coomassie blue.

### **Medium composition**

- MEM
- 6 g/L glucose
- 2.2 g/L NaHCO<sub>3</sub>
- 1% Fetal Calf Serum

### **Culture conditions**

37°C in incubator with 5% CO<sub>2</sub>

### **Ultrapure water systems for media preparation**

System A :

Tap Water feeding Milli RX 12 feeding Milli-Q Plus

System B :

Water from system A processed through double distillation

System C :

Tap Water feeding Milli RX 12 feeding Milli-Q SP UF

## Results

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Figures 1 through 5 show nerve cells cultured in media prepared with water from the various purification systems indicated.

Figure 1 shows results for System A (traditional ultrapure water).

Figure 2 shows the results for System B (ultrapure water double-distilled).

Figure 3 shows results for System C (Milli-Q SP UF water used directly).

In addition, in order to confirm the consistency and repeatability of continuous use of the ultrafiltration membrane, the same experiment was conducted after 3 months of system use and the results (to be compared to Fig 2 and 3) are shown on Figs 4 and 5.

These results show that nerve cells culture is made possible by switching from conventional ultrapure water system to the Milli-Q SP UF system.

For figures 2 to 5, in each case, good nerve cell growth is observed indicating that the water produced by the Milli-Q SP UF system (figures 3 and 5) does not need, as ultrapure water used in figure 1, additional treatment prior use.

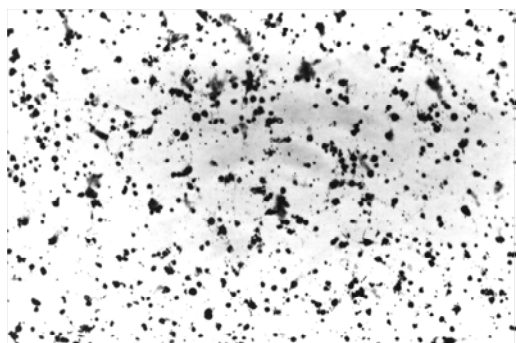


Figure 1. Media prepared with Milli-Q Plus (system A) water shows inhibited cell growth.

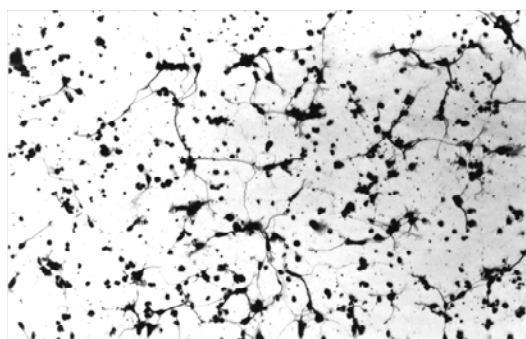


Figure 2. Media via double-distilled Milli-Q Plus water.

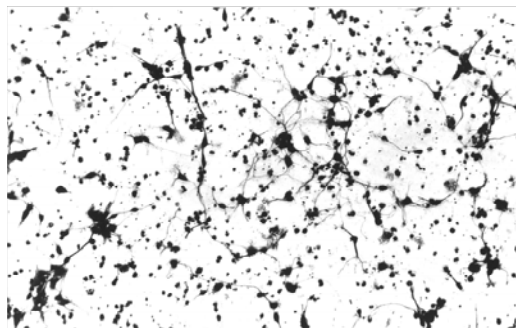


Figure 3. Media using Milli-Q SP UF water.

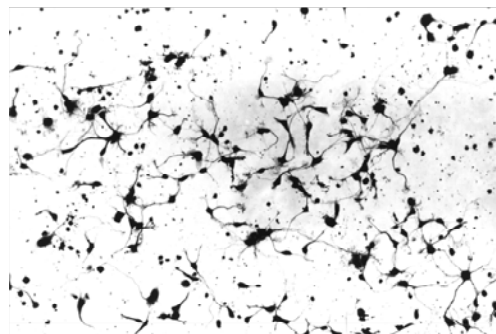


Figure 4. Media using Milli-Q Plus water double distilled 3 months later.

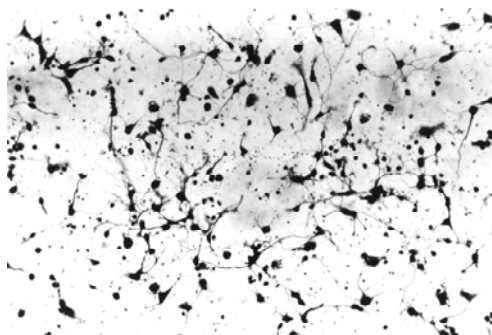


Figure 5. Media using Milli-Q SP UF water 3 months later.

## Conclusions

The ultrapure water produced by the Milli-Q SP UF system allows direct use for nerve cell culture and gives the same high reliability as double-distilled Milli-Q water.

This hollow fiber module, which has a sharply defined molecular weight cut off (5,000 Da), demonstrates superior rejection of pyrogens and organics compared to conventional ultrapure water systems.

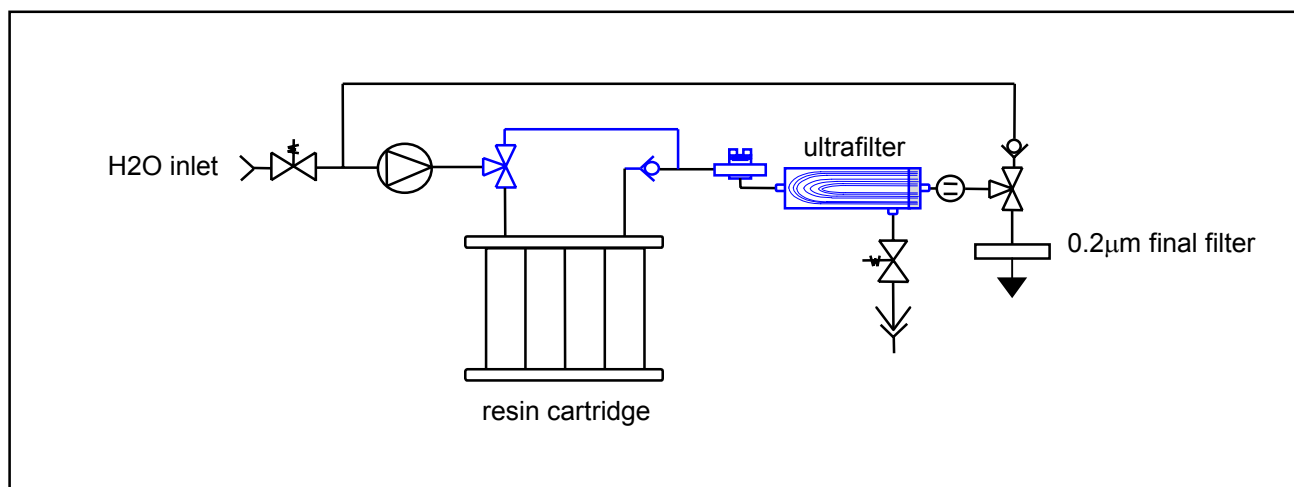
The experiments made three months later (water consumption of 1,500 liters) indicate that the system maintained quality performance to provide pyrogen-free ultrapure water.

The Milli-Q SP UF is the system of choice to produce ultrapure water for experiments such as cell culture, eliminating labor for making pure double-distilled water and contamination from storage.

i Uno K., *Medical Immunology* **Vol 22**, N°22

ii Mori, Matsumoto, Tsukui et al, *Sanpu Sinpo* **Vol 38**, N°1, 1986

**Milli-Q SP UF schematic diagram**



## Part 2 : Qualification of the Milli-Q PF for Pyrogen Removal

Milli-Q PF systems for ultrapure, pyrogen-free water production, are based on the development and design of a new hollow fiber cartridge<sup>1,2,3</sup>.

The production of pyrogen-free water with the Milli Q PF system is based on the validated performance of the included ultrafiltration cartridge having a cut off of 5000 Daltons.

This new UF cartridge contains hollow fibers and operates with 100% product flow recovery. It ensures a 5-log reduction of bacterial contamination (with reverse osmosis pretreatment), less than 3 ml/min air diffusion, and over 1500ml/min flow rate.

### 1. UF cartridge qualification procedure for pyrogen retention

The qualification procedure for the UF cartridges is based on pyrogen challenge tests with the performance of pyrogen reduction being correlated to air diffusion tests. A lot of 21 modules is chosen for the performance tests.

#### 1.1- Pyrogen challenge procedure

Each cartridge was challenged at a low concentration of pyrogen (6 to 10 EU/ml) for 30 minutes and at a high concentration of pyrogen (6,000 to 10,000 EU/ml) for another 30 minutes. Samples from feed and permeate were collected at the end of each concentration of pyrogen challenge, then analyzed via an LAL 5000 endotoxin detection system (Associates of Cape Cod, Falmouth, MA , USA).

#### 1.2 Air diffusion test procedure

The cartridges are wetted with ultrapure water. Air flow diffusion through the UF membrane is measured at 30 psi.

#### 1.3 Results

Obtained results for the air diffusion and pyrogen challenge are reported in the following table.

<u>Module number</u>	<u>Air diffusion</u> ml/min	<u>Pyrogen log</u> high challenge 6000 to 10000,	<u>reduction</u> low challenge 6 to 10 EU/ml
1.1	< 0.05	6.2	3.2
1.2	< 0.05	5.9	3.9
1.3	< 0.05	6.7	3.4
1.4	< 0.05	6.8	3.7
1.5	< 0.05	6.6	3.7
1.6	0.1	6.6	3.2
1.7	0.25	6.7	3.2
1.8	< 0.05	5.7	3
1.9	0.07	6.1	3.6

1.10	<b>0.13</b>	<b>6.6</b>	<b>3.2</b>
1.11	<b>19</b>	<b><u>1.7</u></b>	<b><u>1.5</u></b>
2.1	<b>1.49</b>	<b>5.74</b>	<b>4.7</b>
2.2	<b>1.94</b>	<b>5.53</b>	<b>3.85</b>
2.3	<b>2.6</b>	<b>6.54</b>	<b>4.11</b>
2.4	<b>1.52</b>	<b>6.77</b>	<b>4.34</b>
2.5	<b>1.83</b>	<b>7.25</b>	<b>4.31</b>
2.6	<b>1.83</b>	<b>6.11</b>	<b>4.17</b>
2.7	<b>1.74</b>	<b>5.69</b>	<b>3.57</b>
2.8	<b>1.47</b>	<b>7</b>	<b>4.05</b>
2.9	<b>2.84</b>	<b>7.08</b>	<b>4.17</b>
2.10	<b>2.08</b>	<b>6.66</b>	<b>4.83</b>

**Table 1. Correlation between the air diffusion test and pyrogen reduction**

The results confirm the correlation between the air diffusion test and pyrogen reduction. Among the 21 cartridges tested, one failed to reduce the pyrogen level to the minimum ratio of specification. This failure is reflected in the air diffusion test result which is much higher than the 3 ml/min, maximum allowed. This defective cartridge would have been detected and eliminated by the 100% air diffusion test conducted on each cartridge.

## 2. Other Qualification Tests

For the production of ultrapure, pyrogen-free water, other criteria than pyrogen removal must be considered.

These criteria, in particular, ensure that the UF cartridge is integral and contains low levels of organic or inorganic extractables, and that the procedures used for sanitization are suitable.

Recordings of performance evolution as a function of time were performed on a Milli-Q PF system fed by central RO water.

The tests included:

1. Initial flush-up of the hollow fiber UF cartridge, TOC and resistivity were monitored by Anatel A100 p monitor.
2. Product water quality resistivity and TOC after initial start up.
3. Product water quality after 800 liters of water were withdrawn.
4. Product water quality recovery after sanitization with:
  - chlorine tablet and QPak™ ultrapure water
  - chlorine tablet and RO water
  - 7 grams of NaOH and QPak ultrapure water
  - 7 grams of NaOH and RO water

Performance of the system:

1. At initial flush up, the acceptable 15MΩ-cm value is reached within one hour, and 17MΩ-cm after slightly more than 2 hours
2. After start up, the acceptable 15MΩ-cm value is reached within few minutes, and 17MΩ-cm after approximately 25 minutes (TOC around 15 ppb).
3. After 800 liters of throughput, the resistivity recovery is similar to that observed after start up. The TOC level remains slightly higher at approximately 25 ppb.

4. After sanitization, as a general rule, the resistivity recovery is slightly slower, especially when RO water is used as feed water, except when sanitization is performed with chlorine and Milli-Q feed water.

The TOC level remains essentially unchanged before and after sanitization when RO water is used as feed water. It is even lower after sanitization when feed water is Milli-Q water.

Several Milli-Q PF systems, equipped with water totalizers, were installed for beta site evaluation. Water quality data was collected every two weeks, including resistivity, temperature and flow rate measurements. Samples were also sent for bacteria and pyrogen analysis. All obtained results were within specifications.

### 3. Comments

Results shown in table 1 indicate the high correlation obtained between the air diffusion test and the pyrogen removal efficiency.

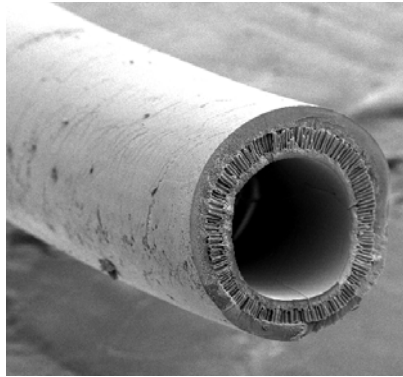
All values, except one, measured for the log reductions are good :in the range log 5.7 to log 7 for “high” concentration tests, and in the range log 3 to log 4.8 for “low” concentration tests.

For the non-satisfactory value, corresponding to the underlined data of module 1.11 in table 1, it is worth noticing that the resulting air diffusion value is also out of specification.

All cartridges are thus 100 % tested for air diffusion, indicative of effective pyrogen removal.

The lot release criteria of the hollow fiber are as follows :

Criteria	Sampling	Acceptance value
air diffusion	100 %	< 3.0 ml/min at 2.0 kg/cm <sup>2</sup>
pyrogen reduction	0.2 %	< 0.01 EU/ml with > 30 EU/ml challenge
resistivity flush up	1%	> 16 MΩ.cm after 15 min rinsing
pressure	100 %	no leakage at 4 kg/cm <sup>2</sup> , closed module
flow rate	100 %	> 1.5 l/min UF permeate at 25 °C
size	1%	length between end caps
outside view	100 %	no visible particle in the module



SEM picture of Ultrafiltration Hollow fiber

#### 4. Conclusion

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The UF cartridge used for pyrogen removal in the Milli-Q PF is, from the best of our knowledge, the only cartridge used in a laboratory water purification system which has been fully qualified for this specific application. Systematic and controlled quality tests with stringent release criteria warranty the reliability of the system.

- <sup>1</sup> Technical Brief Lit N°TB064 *Millipore Corporation*, 9/1994
- <sup>2</sup> Technical Brief Lit N°TB065 *Millipore Corporation*, 9/1994
- <sup>3</sup> Technical Brief Lit N°TB066 *Millipore Corporation*, 3/1995