Application Note: ANWPBACTERIA 0614

Determination of Bacteria Retention in the Thermo Scientific Barnstead GenPure Pro Water Purification System

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Key Words
Bacteria, Ultrapure Water, Thermo Scientific Barnstead, GenPure, ASTM Type 1, E.coli

Abstract
The bacteria retention of a Thermo Scientific™ Barnstead™ GenPure™ Pro water purification system was evaluated using the membrane filtration method based on the European Pharmacopoeia method described in Chapter 2.6.12.[1]

Introduction
Bacteria are single-celled organisms that can be found nearly everywhere in a busy laboratory. Although many of these bacteria are harmless to a person's health, they can create unwelcome variability in an experiment. Because of their abundance and ability to be easily transferred, precautions such as aseptic technique are employed. Using bacteria-free water during steps such as sample preparation, system rinsing, or buffer preparation is an easy method of reducing the chance of bacterial contamination.

Water purification systems are a reliable source for bacteria-free water. On average, bacteria such as *Escherichia coli (E. coli)*, which are widely used in the laboratory, have an length of about 2 µM, and an average diameter of 0.5 µM.[2] A 0.2 µM absolute membrane filter at the end of the system is used to remove any particles or bacteria that are larger than the pore size of the filter.[3] Proper maintenance of the water system, including filter replacement as specified in the manual, helps to ensure the water remains bacteria-free.

Ultrapure water from a Barnstead GenPure water purification system was analyzed for the presence of aerobic bacteria. The GenPure Pro system was chosen from the family of GenPure systems, which also includes the GenPure and GenPure xCAD Plus models. All of these systems have the same feed water requirements, basic flow path and all dispense water through a 0.2 µM final filter. Because these systems require pretreated feed water, the feed water must be treated by reverse osmosis (RO) or deionization (DI) to meet the incoming feed water requirements listed in the system's operational manual[4].

In normal operation, the acceptable incoming bacteria concentration for the system's feed water is defined as <100 CFU/ml. To challenge the system, the GenPure Pro system was connected to a tap water line rather than its normal pre-treated feed water, to help determine if the increased bio-load would impact the system's ability to retain the bacteria.

Materials and Methods
Bacteria determination of the GenPure Pro system using pre-treated feed water

As stated in the introduction, the GenPure Pro system is an ultrapure water system, which requires pre-treated water, otherwise known as a polisher system. To pre-treat the feed water for the GenPure Pro system, a Thermo Scientific™ Barnstead™ Pacific™ TII 20 UV water purification system was set up according to the operational manual[5], along with a 60 L Thermo Scientific reservoir, as demonstrated in Figure 1. The system was put into operation and 70 L of water was dispensed from the storage reservoir and discarded. The GenPure Pro was then connected to the reservoir and rinsed with 10 L of water.

Water sampled from the 60 L storage tank, as well as the ultrapure water produced from the GenPure Pro system, were tested for bacterial growth using the membrane filtration technique[1]. Clean techniques were used to reduce the chance of environmental contamination.

After dispensing 0.2 L of water from the reservoir and GenPure Pro system, 1 L samples were collected in sterile flasks at the outlet of the storage reservoir and the GenPure Pro system to probe the water qualities.

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Figure 1. Diagram of a Pacific TII water system with a 60 L reservoir feeding a GenPure Pro water system.

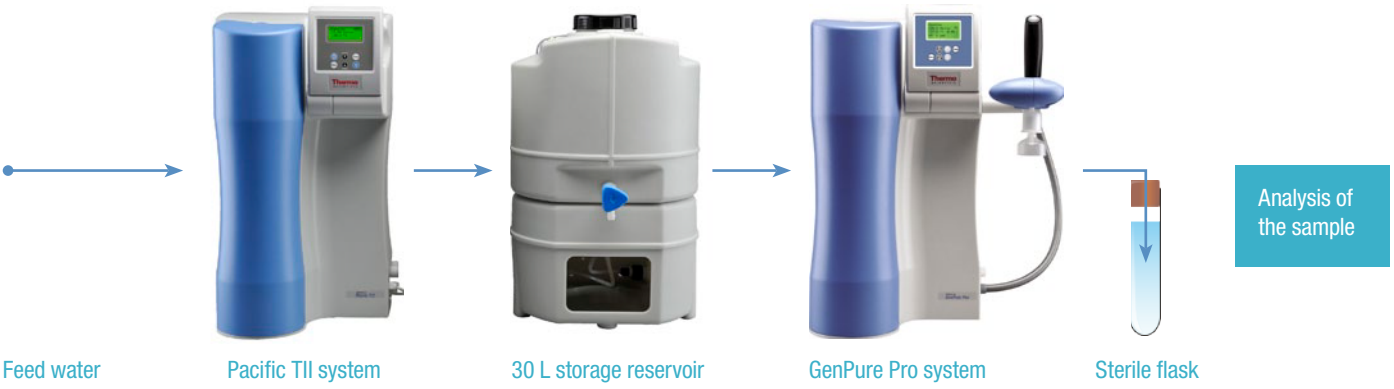

Figure 2. Diagram of the set-up for the GenPure Pro water system being fed tap water

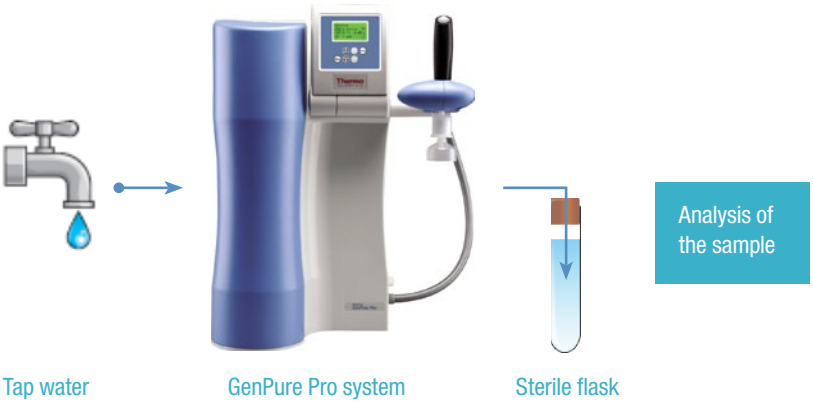

Figure 3. Pictures of the CN membrane treated with tap water (left) and treated with ultrapure water produced from the GenPure Pro system fed with tap water (right)

The complete 1 L sample was filtered through a 0.2 µm cellulose nitrate (CN) membrane. The membrane was then transferred aseptically to a R2A-Agar and incubated at 35° C for 5 days in a Thermo Scientific™ Heratherm™ compact microbiological incubator (model IMC18). The entire procedure was repeated after all consumables in the systems and reservoirs were replaced with new ones to produce two sets of data.

Bacteria determination of the GenPure Pro system using tap feed water

The previously tested GenPure Pro system was disconnected from the Pacific TII system and 60 L reservoir and connected to tap water to create a new feed water source, as shown in Figure 2.

To equilibrate the system on tap water, 30 L of tap water was rinsed through the GenPure Pro system and then 1 L samples were collected at the inlet and outlet of the GenPure Pro system to probe the bacteria concentration for both the feed and product water. The 1 L sample was filtered through a 0.2 µm cellulose nitrate (CN) membrane, which was then transferred aseptically to a R2A-Agar and incubated at 35° C for 5 days in a Heratherm compact microbiological incubator (model IMC18). After 5 days, the colonies were counted for all 4 samples and the amount of bacteria per ml (CFU/ml) in the feed and product water was calculated. The entire procedure was repeated after all consumables in the systems were replaced with new ones to produce two sets of data, for a total of 8 samples.

Results

After 5 days, any bacteria colonies found on the R2A-Agar plates were counted (as shown in Figure 3) and the amount of bacteria per ml in the water were calculated. The data is summarized in Tables 1 and 2.

Table 1: Bacteria count from the storage reservoir and the GenPure Pro system

	Storage Reservoir Water – Run 1	Storage Reservoir Water – Run 2	GenPure Water – Run 1	GenPure Water – Run 2
Bacteria CFU/ml	>10	> 10	<0.01	<0.01

Table 2: Bacteria count from the tap water and from the GenPure Pro system

	Tap Water – Run 1	Tap Water Water – Run 2	GenPure Water – Run 1	GenPure Water – Run 2
Bacteria CFU/ml	>10	> 10	<0.01	<0.01

Conclusion

The GenPure Pro system was able to filter bacteria down to < 0.01 CFU/ml, even when challenged to purify tap water.

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Trace Metal Analysis of Water Produced From a Thermo Scientific Barnstead GenPure UV/UF-TOC Water Purification System

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Key Words
ICP-MS, IC, Ultrapure Water, Thermo Scientific Barnstead, GenPure, Ions, ASTM Type 1

Abstract
Laboratory water produced from a Thermo Scientific™ Barnstead™ GenPure™ UV/UF-TOC water purification system was analyzed for 40 elements by M. Reutz and R. Braitmayer at Analytik für Technik und Umwelt GmbH.

Introduction
Effective and repeatable purification is critical for the production of ultrapure water with trace levels of ions, which is necessary in many analytical procedures. Although ultrapure water, which measures 18.2 Megohm·cm, is theoretically ion-free, it may still contain parts per trillion (ppt) levels of ionic species. For many scientific applications, these trace levels of ions are below detection limits and will not interfere with their results. As modern analytical instrumentation continues to push the boundaries of ultralow sensitivities, allowing for the elemental analysis of samples at levels of parts per trillion, extreme care must be taken to ensure that the sample is not contaminated during preparation, storage or handling.

An ASTM[1] Type 1 water purification system is only required to produce water that is 18.0 Megohm·cm and achieve <50 ppb total organic carbon (TOC) per the standard. As analytical applications become more sensitive to ppt levels of ions, water systems have become more advanced in their ability to remove trace ions.

To help ensure the ultrapure water produced by the Barnstead GenPure water system has ultralow levels of ions, the water system employs several purification technologies as the ions could be simply dissolved in the water or bound as a ligand with organics in the feed water (Figure 1). As the feed water enters the system, it is exposed to an ultraviolet (UV) lamp. The dual wavelength lamp (185/254 nm) will oxidize any organic impurities in the feed water, releasing any metal ligands that could be present.

The next purification step is the ultrapure cartridge, where ionic impurities are sequestered in the ion exchange resin. The cartridge in the GenPure UV/UF-TOC system uses high-quality ion exchange resins (semiconductor grade) to effectively and consistently produce ultrapure water with a resistivity of 18.2 Megohm·cm and a TOC of <5 parts per billion (ppb).

Additionally, the wetted parts in the water system are constructed with high-purity materials including virgin polypropylene and high-purity fluoropolymer delivery components to reduce contamination from the system itself.

Water from a GenPure UV/UF-TOC system was analyzed using validated trace element techniques in a clean room environment. Inductively coupled plasma mass spectrometry (ICP-MS) and ion chromatography (IC) were used to analyze the water for trace metal and ion contamination. The water was tested for 40 ionic impurities.

Materials and Methods
Performed by: M. Reutz and R. Braitmayer, Analytik für Technik und Umwelt GmbH, Herrenberg, Germany

A GenPure UV/UF-TOC system was fed with deionized (DI) water in a clean room. The water feeding the GenPure system was from the laboratory's central water system and exceeded the minimum feed water requirements as described in the GenPure system's operational manual[2]. The system was initially rinsed with DI water for two days prior to sampling the water for analysis. All sampling was performed using clean techniques. Anions and cations were analyzed using a Thermo Scientific™ Dionex™ model IC 20 ion chromatograph. Trace metals were analyzed using Agilent™ model ICP-MS 7500cs. Because of interferences using ICP-MS, silica was analyzed by a PerkinElmer™ model GF-AAS 3030.

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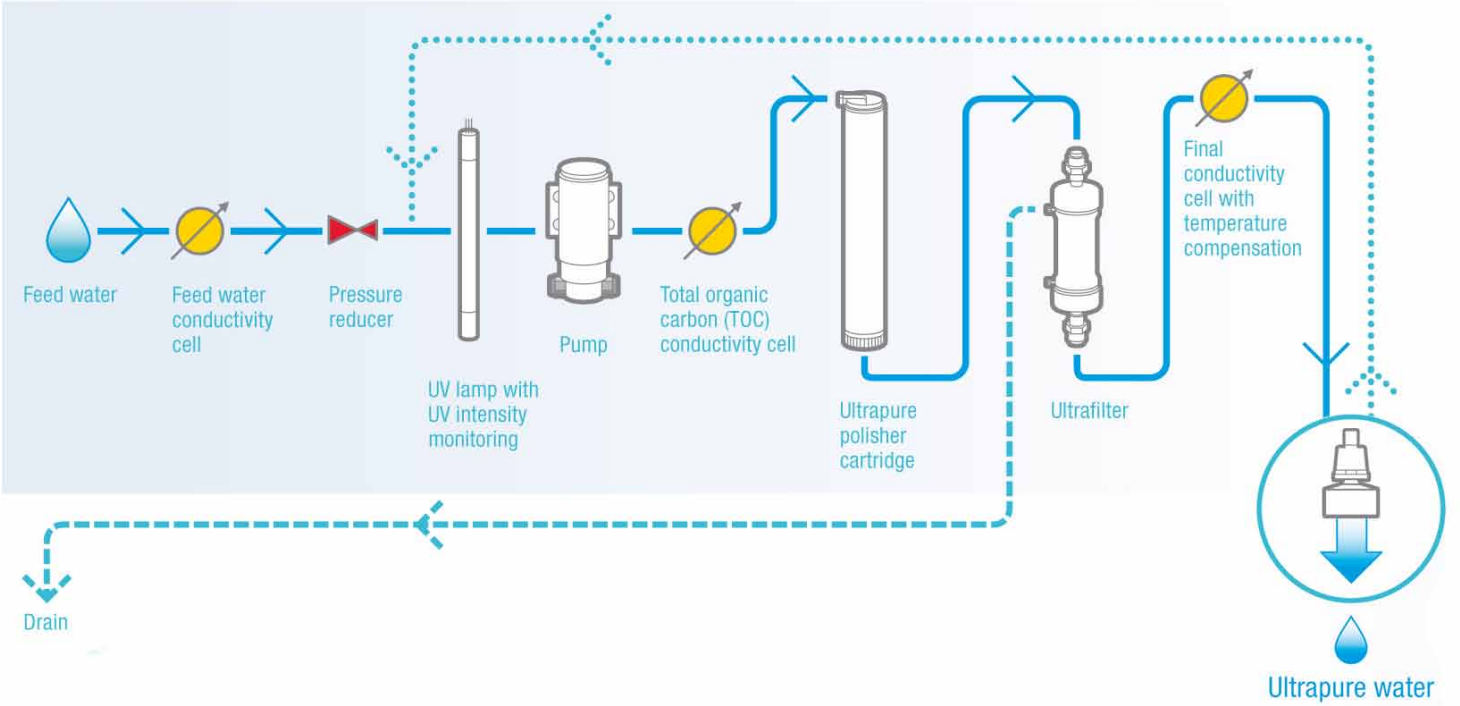
Figure 1. Flow diagram of a GenPure UV/UF-TOC water system.



Results

Tables 1 and 3 list the levels of ionic species determined in the product water of a GenPure UV/UF-TOC water system. Of the 40 elements and ions tested, 37 were below the detection level of the system. Three elements — calcium, sodium, and zinc — demonstrated an increase in their amount when their levels in the feed and product water were compared. It is possible that the ultrafilter, which is one of the last purification steps in the water system, could be introducing these impurities as it is located after the ion exchange cartridge, but the data set does not address this question. Because of this risk, ultrapure water systems configured with only a UV lamp are recommended for sensitive applications, whereas laboratories doing molecular or cellular biology experiments are recommended to configure their systems with a UV lamp and ultrafilter to remove pyrogens and nucleases. Additionally, some of the elements had no detectable levels of ions in the feed water. Assessing the levels of compounds in the water produced by a water purification system without some measurable level in the feed water demonstrates that the system, as configured, did not introduce contamination, but does not indicate whether the system has the ability to remove these impurities.

Table 2 lists the measured TOC of the system's product water, which is within the published specifications of the system.

References

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Table 1: Ion analysis of the feed and product water of a GenPure UV/UF-TOC water purification system using ion chromatography.

Parameter	Detection Limit, ppt	Results, ppt
Ammonium NH^{4+}	10	< 10
Fluoride F-	5	< 5
Chloride Cl-	5	< 5
Bromide Br-	5	< 5
Nitrite NO^{2-}	5	< 5
Nitrate NO^{3-}	5	< 5
Phosphate PO_4^{3-}	20	< 20
Sulfate SO_4^{2-}	10	< 10

Table 2: Determination of the TOC level in the feed and product water of a GenPure UV/UF-TOC system using ICP-MS

Parameter	Detection Limit, ppt	Results, ppt	
		Feed Water	Product Water
TOC	0.1	6.5	2.0

Table 3: Results from the analysis of the feed and product water of a Barnstead GenPure UV/UF-TOC water purification system.

Element	Detection Limit, ppt	Results, ppt		Method
		Feed Water	Product Water	
Aluminium Al	0.3	< 0.3	< 0.3	ICP-MS
Antimony Sb	0.5	< 0.5	< 0.5	ICP-MS
Arsenic As	0.5	< 0.5	< 0.5	ICP-MS
Barium Ba	0.1	< 0.1	< 0.1	ICP-MS/IC
Bismuth Bi	0.5	0.6	< 0.5	ICP-MS
Boron B	20	560	< 20	ICP-MS
Cadmium Cd	0.5	< 0.5	< 0.5	ICP-MS
Calcium Ca	1	< 1	2.8	ICP-MS/IC
Chromium Cr	0.2	0.6	< 0.2	ICP-MS
Cobalt Co	1	1.2	< 1	ICP-MS
Copper Cu	0.5	< 0.5	< 0.5	ICP-MS
Iron Fe	0.5	2.4	< 0.5	ICP-MS
Lead Pb	0.2	2.5	< 0.2	ICP-MS
Lithium Li	0.1	0.2	< 0.1	ICP-MS/IC
Magnesium Mg	0.1	0.2	< 0.1	ICP-MS/IC
Manganese Mn	0.5	2.8	< 0.5	ICP-MS
Molybdenum Mo	0.2	0.6	< 0.2	ICP-MS
Nickel Ni	0.2	0.7	< 0.2	ICP-MS
Palladium Pd	0.2	< 0.2	< 0.2	ICP-MS
Platinum Pt	1	< 1	< 1	ICP-MS
Potassium K	0.5	1.0	< 0.5	ICP-MS/IC
Silicon Si	200	Not determined	< 200	GF-AAS
Silver Ag	0.2	< 0.2	< 0.2	ICP-MS
Sodium Na	0.1	0.3	0.6	ICP-MS/IC
Strontium Sr	0.2	< 0.2	< 0.2	ICP-MS/IC
Tantalum Ta	0.2	< 0.2	< 0.2	ICP-MS
Tin Sn	1	< 1	< 1	ICP-MS
Titanium Ti	0.2	< 0.2	< 0.2	ICP-MS
Tungsten W	0.5	< 0.5	< 0.5	ICP-MS
Vanadium V	0.2	< 0.2	< 0.2	ICP-MS
Zinc Zn	0.5	1.7	17	ICP-MS
Zirconium Zr	0.2	< 0.2	< 0.2	ICP-MS

On-demand Nuclease- and Endotoxin-Free Lab Water Using a Thermo Scientific Barnstead GenPure UV/UF Water Purification System

Application Note

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Key Words
Water purification, lab water, ultrapure water, nuclease, endotoxin, pyrogen, RNase, DNase, Thermo Scientific Barnstead, GenPure Pro

Abstract
A Thermo Scientific™ Barnstead™ GenPure™ Pro UV/UF water purification system was challenged with RNase, DNase and endotoxins to evaluate the effectiveness of its ability to reduce these impurities below detectable limits and produce nuclease- and endotoxin-free ultrapure water.

Introduction
Nuclease is the general name which includes both ribonuclease (RNase) and deoxyribonuclease (DNase), the enzymes responsible for degrading RNA and DNA respectively. In controlled experiments, these enzymes can be very beneficial as they are used in many life science experiments to cleave specific links on RNA and DNA strands. In contrast, nucleases can also be detrimental to experiments if they are present in applications that require the RNA or DNA to be whole. Controlling nuclease contamination can be a challenge, but it is necessary for accurate and reproducible results in these types of experiments. Very durable, nucleases are resistant to heating, are active over a wide pH range, re-nature readily and are easily transferable.[1] They can be plentiful on counter tops, centrifuges, laboratory glassware, buffer and reagent solutions. They can even be found on gloved hands that have touched hair or skin.

It is important to reduce possible contamination of nucleases, and there are multiple ways to achieve this. Using nuclease-free water for buffers and reagents is a good first step. Traditional practices include inactivating RNase in water with the use of the inhibitor Diethyl Pyrocarbonate (DEPC) followed by autoclaving the water to destroy the inhibitor. DEPC hydrolyzes when exposed to trace levels of moisture, so proper storage requires a layer of inert gas in the bottle after each use. DEPC can only be used with glass pipettes as it will dissolve some plastics and is not recommended to be used with common buffers such as Tris. Lastly, if exposed to ammonia, DEPC can decompose to a possible carcinogen, urethane.[2] Alternatively, bottled nuclease-free water is also available, but this adds more consumables to manage, with the additional risk of contaminating the bottle during each use, taking time and resources away from valuable research.

Just as nucleases can be detrimental to many life science experiments, so can endotoxins. Endotoxins are lipopolysaccharides in gram negative bacteria, which are left behind during the course of the bacteria's life cycle. Endotoxins (also referred to as pyrogens), can induce a high fever when injected into mammals. When present in vitro, endotoxins can interfere with the growth of tissue cultures. To utilize water that is endotoxin-free, many labs utilize endotoxin-free bottled water. While this can be convenient, the bottle can also become contaminated and is another consumable that needs to be ordered, shipped, and stored.

Point of use ultrapure water purification systems with ultrafiltration (UF) are designed to effectively reduce nuclease and endotoxin macromolecules to below detection limits. Ultrafilters used in Thermo Scientific water purification systems use polysulfone hollow fibers to provide a powerful and consistent barrier to trap these particles. In the Barnstead GenPure Pro UV/UF system this filter is strategically placed in-line, at the end of the water system's flow path, to help ensure the complete elimination of all nucleases and endotoxins without possible outside contamination. Proper maintenance of the water system, such as regular system cleaning and prompt filter replacement as specified in the operational manual, helps to ensure the ultrapure water remains contaminate free.

Systems also incorporating an ultraviolet (UV) light with an ultrafilter create a powerful component to further purify the water. A dual wavelength UV light uses its 185 nm wavelength to reduce total organic carbon (TOC) levels to 1 - 5 ppb, and its 254 nm wavelength to maintain an

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aseptic environment as the water is circulated throughout the system.[3] A GenPure water system with UV and UF is designed to effectively deliver high quality, ultrapure water on demand with ultralow TOCs, and free of bacteria, nuclease and endotoxin contaminants. This allows for efficient work flow, and productive use of resources and space.

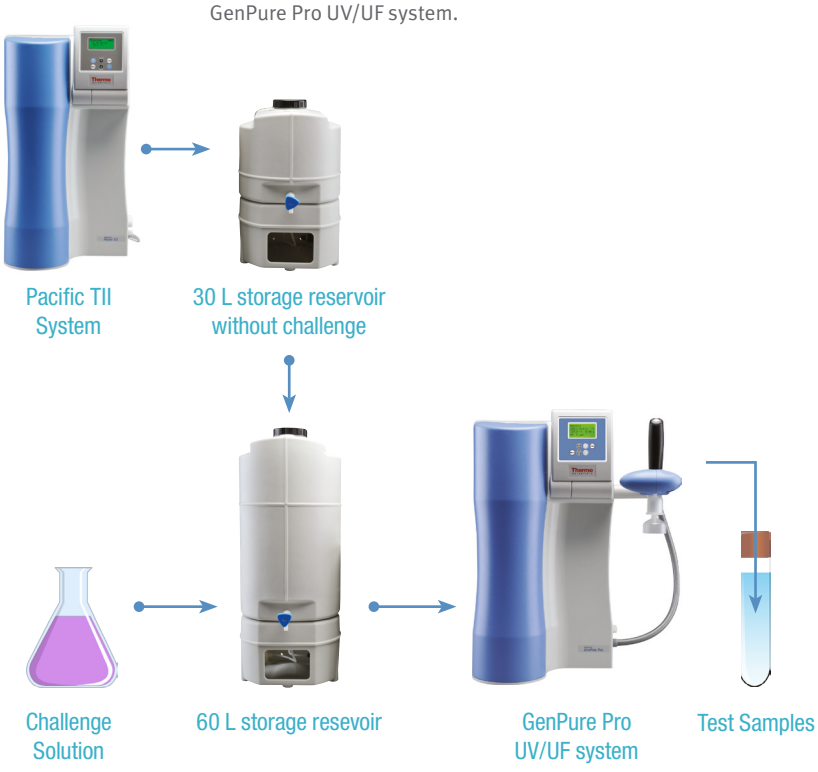
A Barnstead GenPure Pro UV/UF water system was challenged with RNase A and DNase I, nucleases commonly used to qualify ultrapure water systems for nuclease reduction. The GenPure Pro UV/UF system was also challenged with *E. coli* O55:B5 endotoxin. This system was chosen from the family of Barnstead GenPure systems, which also includes the GenPure UV/UF and GenPure xCAD Plus UV/UF models. All of these systems have the same feed water requirements, basic water flow pattern, ultrafilter filtration, UV lamp and dispense water through a 0.2 µM final filter. To challenge the system, the GenPure Pro UV/UF system was connected to a storage tank with a high concentration of solution containing RNase, DNase, and endotoxins to determine if the increased bio-load would impact the system's ability to reduce these impurities.

Methods

Nuclease and endotoxin performance testing in GenPure Pro UV/UF system

The GenPure Pro UV/UF system is an ultrapure water system, which requires ASTM Type II pre-treated feed water. A Thermo Scientific™ Barnstead™ Pacific™ TII 20 UV system with a 30 L Thermo Scientific reservoir was used to pre-treat tap water and was set up according to the operational manual.[4] A 60 L Thermo Scientific storage reservoir was set up to directly feed water into the GenPure Pro UV/UF system to introduce known challenge solutions and the GenPure Pro UV/UF system was set up per its operation manual.[4] The systems were set up as demonstrated in Figure 1.

Figure 1. Diagram of a Pacific TII system with a 30 L reservoir without challenge, 60 L reservoir for challenge solutions and GenPure Pro UV/UF system.



Clean techniques were used throughout to reduce the chance of nuclease or endotoxin contamination. Three samples were routinely collected so that one sample was sent for nuclease analysis, one for endotoxin analysis, and one was archived to protect against shipping errors. The samples were stored at -20°C until they were analyzed.

Negative Controls:

A 60 L reservoir was filled with 15 L of Pacific TII system product water and 10 L was rinsed through the GenPure Pro UV/UF system without the 0.2 µM final filter, followed by 1 L rinse with the 0.2 µM final filter. After a 0.2 L rinse from the 60 L reservoir spigot, three 10 ml samples were taken directly from the reservoir to determine the nuclease and endotoxin levels of the water feeding the GenPure Pro UV/UF system (see "Feed Water" in Table 1). After 0.2 L of water was dispensed from the GenPure Pro system, three 10 ml samples were collected to establish a non-challenged baseline ("Pre-Challenge Water" samples listed in Table 1).

Nuclease Challenge Protocol

A challenge solution with 1 µg/mL RNase and 100 U/L DNase was prepared by adding 500 µL of 10 mg/mL RNase A stock solution and 110 µL of 4.5 U/µL DNase to 5 L of UltraPure™ DNase/RNase-Free distilled bottled water from Life Technologies. The 60 L storage reservoir was drained of remaining water, and the challenge solution was introduced to the reservoir that was connected to the GenPure Pro UV/UF system. Water was dispensed continuously from the GenPure Pro UV/UF system dispenser and three 10 mL samples were taken at specific volume intervals: 2.5 L, 5 L, 10 L, 20 L, 30 L, 40 L, and 50 L. The Pacific TII system was used to replenish water in the 60 L challenge reservoir to complete the sampling. After collecting all samples, the GenPure Pro UV/UF system was sanitized and consumables changed per the system's operational manual[5] and the entire procedure was repeated to create the run 2 data set. Samples were shipped to Thermo Fisher Scientific Baltics UAB, Lithuania for analysis.

The RNase analysis was performed by incubation of 80 ng of 2 kb RNA transcript for 4 hours at 37°C with 8.2 µL of the water sample in RNase assay buffer with Mg^{2+}, in a 20 µL total reaction mixture. After incubation, the integrity of RNA was analyzed on a 1% agarose gel and stained with ethidium bromide. RNase contamination is not detectable with a detection limit of 1 x 10^{-7} Unit per reaction (0.003 ng/mL). The data for the RNase challenge is in Table 1.

DNase testing was conducted by incubation of 1.2 µg of supercoiled pUC19 DNA/SmaI with 15.6 µL of the water sample in DNase assay buffer with Mg^{2+} for 17 hours at 37°C, a 24 µL total reaction mixture. After incubation the DNA was analyzed on 1% agarose gel and stained with ethidium bromide. DNase is not detectable with detection limit of 1 x 10^{-6} Unit per reaction (0.002 pg/µL). The data for the DNase challenge is in Table 1.

Endotoxin Challenge of the GenPure Pro UV/UF system

A challenge solution was prepared adding 5 vials of 1,250,000 EU/vial *E.coli* O55:B5 Endotoxin to 5 L of

Table 1. RNase, DNase, and endotoxin detection in ultrapure water produced by a Barnstead GenPure Pro UV/UF water purification system. Data for both runs were identical unless noted otherwise.

	RNase Concentration (ng/mL)	DNase Concentration (pg/µL)	Endotoxin Concentration (EU/mL)	
Feed Water	<0.003	<0.002	Run 1: 0.112	Run 2: 0.0271
Pre-challenge water	<0.003	<0.002	<0.001	
2.5 L post challenge	<0.003	<0.002	Run 1: <0.002*	Run 2: <0.001
5 L post challenge	<0.003	<0.002	<0.001	
10 L post challenge	<0.003	<0.002	<0.001	
20 L post challenge	<0.003	<0.002	<0.001	
30 L post challenge	<0.003	<0.002	<0.001	
40 L post challenge	<0.003	<0.002	<0.001	
50 L post challenge	<0.003	<0.002	<0.001	

* Unknown interference in sample was detected. Sample was diluted and retested with different detection limit.

UltraPure™ DNase/RNase-Free distilled bottled water. The challenge solution was introduced to the 60 L reservoir that was connected to the GenPure Pro UV/UF system. Water was dispensed and sampled as above in the RNase/DNase challenge. The endotoxin analysis was conducted by Nelson Laboratory, Salt Lake City, UT. Samples were analyzed using the Bacterial Endotoxins Test: Kinetic Chromogenic Method or Limulus Amebocyte Lysate (LAL) test to detect and quantify bacterial endotoxin. Endotoxin was not detectable with detection limit of 0.001 EU/mL. The data for the endotoxin challenge is in Table 1.

Results

Table 1 lists results from runs 1 and 2 for the RNase, DNase and endotoxin analysis. For results that were identical in both runs, only one result is reported. RNase and DNase levels were below detection limits in the Pacific TII 60 L tank, which is listed in the table as feed water. The pre-challenge water samples in the table refer to the samples collected from water dispensed from the GenPure Pro UV/UF system before the challenge solution was introduced. Here again, the levels were below the detection limit. After the challenge solution was introduced into the feed water for the GenPure Pro UV/UF system, samples were taken at timed intervals. All post-challenge samples were determined to be below the RNase/DNase detection limits.

The endotoxin analysis, on the other hand, indicated endotoxins were already present in the feed water to the GenPure system even before the system was challenged. The pre-challenge water sample was below the level of detection, so any endotoxins naturally present in the feed water was reduced by the system. All endotoxin levels in samples taken at specific volume intervals were found to be below the level of detection, and one sample at 2.5 L contained an unknown interference in the analysis, so the sample had to be diluted and retested with a result reporting as a different detection limit than other data points.

Conclusion

In-line ultrafiltration combined with UV oxidation provided an easy and efficient method of reducing nucleases and endotoxins from water below detectable limits, even when the Barnstead GenPure Pro UV/UF water purification system was challenged with 5mg RNase A, 500 U DNase I, and 6,250,000 EU *E.coli* O55:B5 endotoxin.

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