Determination of Bacteria Retention in the Thermo Scientific Barnstead Smart2Pure Water Purification System

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Key Words

Water purification, lab water, ultrapure water, bacteria, 0.2 micron filter, Thermo Scientific Barnstead, Smart2Pure

Abstract

The bacteria retention of a Thermo Scientific™ Barnstead™ Smart2Pure™ 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 sterile technique were 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 Smart2Pure water system was analyzed for the presence of aerobic bacteria. The Barnstead Smart2Pure 3 system was chosen from the family of Smart2Pure systems, which also includes Smart2Pure UV and UF 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. The systems are fed by tap water and utilize two purification steps to create 18.2 megohm lab water. The first step purifies the feed water using a reverse osmosis (RO) membrane to remove the majority of impurities in the water. The second step of purification utilizes an ultrapure cartridge to remove any ions in the water. Additionally, an optional ultraviolet (UV) lamp and/or an ultrafilter (UF) can be added to the system configuration.

Methods

Bacteria retention of the Smart2Pure system using tap water as feed water

The bacterial content of tap water was determined after disinfecting the water outlet with 70% ethanol and rinsing with 1 L water. Afterwards three 1 L sample volumes were collected and tested for bacterial growth using the membrane filtration technique.[1] Three different volume samples were generated from the three 1 L samples: a 1 L, 10 mL, and a 1 mL sample. These three samples were filtered through a 0.2 µm cellulose nitrate (CN) membrane. The membrane was afterwards 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 agar plates with sample volumes of the membrane filtration (1 L and 10 ml) showed after 5 days incubation at 35°C too many colonies to count. Only the 1 ml sample volumes gave results that could be quantitated.

After installing and rinsing of the Smart2Pure 3 system according to the Smart2Pure water purification system operating instructions, the system was ready for sampling. 0.2 L of water from the system was used to rinse the 0.2 µm filter of the Smart2Pure 3 system and then 1 L samples were collected in sterile flasks.

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Figure 1. Diagram of a Smart2Pure water system with a 6 L internal reservoir.

Tap water   Smart2Pure 3 system   Sterile tube

Analysis of the samples

Figure 2. Set up of the filtering of the 1 L sample, using a Thermo Scientific Nalgene analytical test filter funnel, through the 0.2 μm cellulose nitrate (CN) membrane.
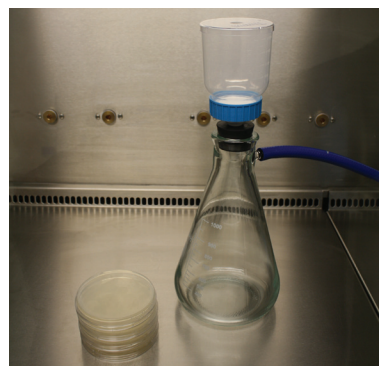

The 1 L sample was then filtered through a 0.2 μm cellulose nitrate (CN) membrane, using a Thermo Scientific™ Nalgene™ analytical test filter funnel, as shown in Figure 2. The membrane was afterwards transferred aseptically to a R2A-Agar and incubated at 35°C for 5 days in a Heratherm compact microbiological incubator (model IMC18). The Smart2Pure 3 system was disinfected with the disinfection routine according to operating instructions, and the pretreatment cartridge, ultrapure polisher cartridge and 0.2 μm filter were replaced with new ones to retest the system.

Results
After 5 days, any bacteria colonies found on the R2A-Agar plates were counted and the amount of bacteria per ml in the water were calculated. The data is summarized in Table 1.

Conclusion
The Smart2Pure system, which was directly connected to tap water, was able to remove the bacterial load of the tap water down to < 0.01 CFU/ml.

References
1. European Directorate for the Quality of Medicines & HealthCare (EDQM): European Pharmacopoeia 6.8, *Microbiological Examination of Non-sterile Products: Microbial Enumeration Tests*, 2010

2. Thermo Scientific Waterbook. 2014. Thermo Fisher Scientific. Retrieved 12 June, 2014 from http://www.thermoscientific.com/purewater

3. Operational Instructions for the Thermo Scientific Barnstead Smart2Pure water purification system. Thermo Fisher Scientific, Niederelbert, Germany.

Table 1. Bacteria count from the tap water line (1 L and 10 ml)

	Tap Water Sample (10 ml)	Smart2Pure System Sample (1 L)
1st Run		
2nd Run		

	Tap Water	Smart2Pure System Water
Bacteria Count (CFU/ml)	n.a.	<0.01

n.a. not applicable, the colonies were too numerous to evaluate

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