# Validation Guide

Medical Filters

Pentair Filtrix

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# 1. Introduction

From the water purification plant to the point of use water passes a variety of piping and distribution systems. Although initially the microbial load of at the outlet of the plant is relatively small, at the end of this chain a high microbial count can be found. Many of these microorganisms are harmless, but opportunistic pathogens like *Pseudomonas*, *Legionella* and fungi have been found. Microorganisms can accumulate through accumulation on surfaces and growth in a so-called biofilm. These biofilms are very difficult to remove by chemical or heat treatments and regularly release microorganisms in the water for further colonization. From the water phase opportunistic pathogens can reach humans via drinking, inhalation of aerosols and bathing. This, in turn, can lead to infections and diseases like Legionellosis.

Pentair Filtrix produces Medical Water Filters that contain capillary microfiltration membranes with a pore size of 0.2 micron, which effectively retain bacteria and fungi. While water molecules pass through the porous wall of these hollow fiber membranes, the pores retain micro-organisms and other particular contaminants. The Pentair Medical Filters provide easy and reliable protection at the last possible moment before patient contact.

This validation guide summarizes tests that have been performed for validation and qualification of the Pentair Filtrix Medical ShowerFilters and TapFilters.

All tests have been performed with regular off-the-shelve products that have been treated with by gamma irradiation with a minimum dose of 25 kGy.

# 2. Microbiological tests

## *2.1 Retention of Pseudonomas dimunita*

Membranes retain all particles that are larger than their pores and allow passage of water and smaller particles. Thus retention of a small bacterium should be evaluated as a worst case scenario. Testing with the small bacterium *Pseudonomas dimunita* was performed by Vitens laboratory, the Netherlands, an ISO 17025 accredited lab, according to ASTM F 838-05. Membranes were challenged with a high microbial load of at least $6 \times 10^9$ bacteria per L and effluent microbial concentrations were measured. No bacteria were detected in effluent samples resulting in a reduction of at least log 6, the international standard for microbial water purifiers.

## *2.2 Microbial retention over the lifetime*

### 2.2.1 Test description

To test the microbial retention over the lifetime of the filter a dedicated setup was developed and test were performed based on the NSF protocol P231 protocol for microbial water purifiers. Membranes were challenged with a high microbial load three times per week over a period over 35 days. Effluent microbial concentrations were measured and compared to influent concentration to determine the log reduction. Tests were performed on the reference bacterium *Klebsiella terrigena*, the clinically relevant *Legionella pneumophila* and the fungi *Aspergillus fumigatus.*

### 2.2.2 Test results

For both *Klebsiella terrigena* and *Legionella pneumophila* a reduction of more than log 6 was obtained for the complete 35 days, compliant with international standards. Furthermore, no *Aspergillus fumigatus* was detected in the effluent samples.

### 2.2.3 Conclusions

Microbial retention was shown for both bacteria and fungi over a period of 35 days.

*Management summaries of retentions tests issued by Vitens laboratory are added as an appendix.*

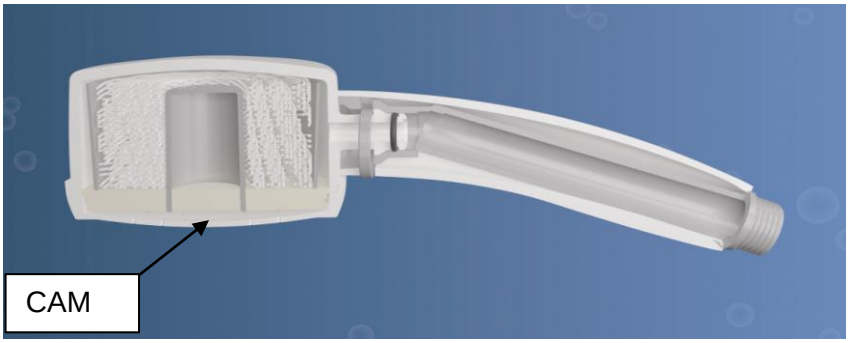
## *2.3 Clinical tests*

Clinical test were performed in a hospital with an increased *Legionella* count in water from showers. Results obtained during weekly tests over 35 days showed that 23 of 24 water samples from showers from several departments contained *Legionella,* while *Legionella* count in all water samples from the Medical ShowerFilter were below the detection limit. It was concluded that also in the clinical setting microorganisms are completely retained by Medical Filters.

*Management summaries of the clinical test issued by Vitens laboratory is added as an appendix.*

## *2.4 Evaluation of Medical Filters with antimicrobial additives*

Next to retention of bacteria on the influent side of the membrane there is also the risk of growth of bacteria on the membrane housing, at the effluent side of the membrane. Although all bacteria are removed from the water supply by the membranes, bacteria from the atmosphere can get into the water-filled compartment after the membranes (CAM) and start to grow over time. Bacteria are known to stick to these plastic surfaces and form a biofilm. This is generally known as cross-contamination.




Based on an extensive study with different types of antimicrobials we have introduced a product line with antimicrobials. In these products the plastic of the spray cap is blended with a polymer additive containing silver. Below is an evaluation of these testing performed on these products.

### 2.4.1 Test set-up

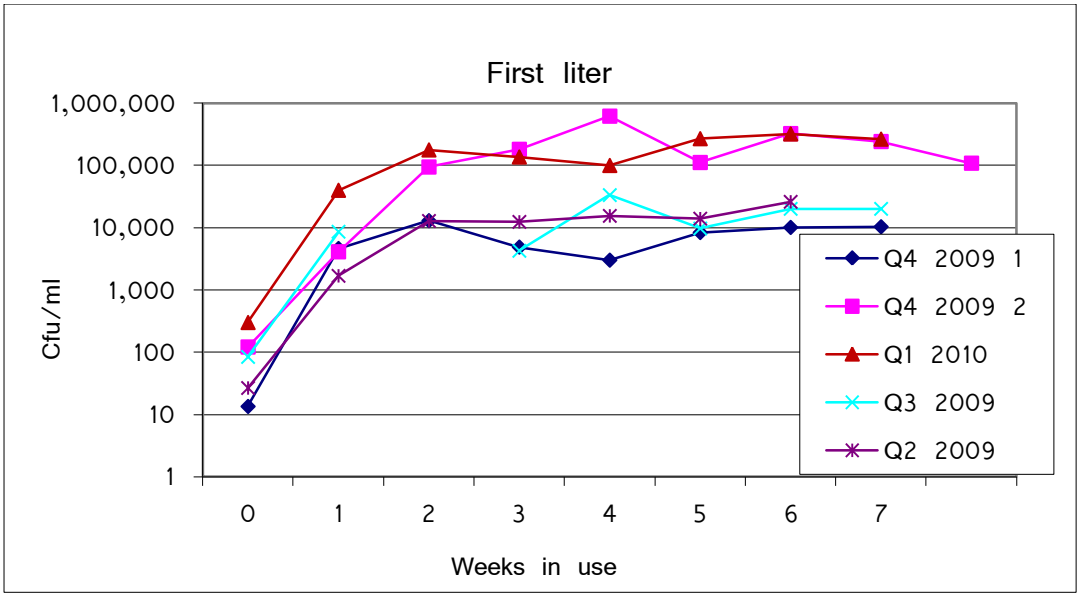
The tests were performed using a so called simulated shower setup. The Medical Filters were placed in a test rig at our facility in the Netherlands, at ambient temperatures and in a frequently used room, to simulate shower or tap situation in a hospital room. The total test was conducted over a period of 8 weeks. All tests were performed in triplicate and results were averaged.

Samples were analyzed for heterotrophic bacteria by Vitens, Laboratories, Leeuwarden. The so called R2A method conform NEN 6276 was used, which uses plates with R2A medium and an incubation over 10 days at 25 ºC. After incubation the total amount of colony forming units (CFU) is determined and used to calculate the amount of CFU/ml in the sample. The R2A method is considered a very sensitive method for determining heterotrophic bacteria in water samples.

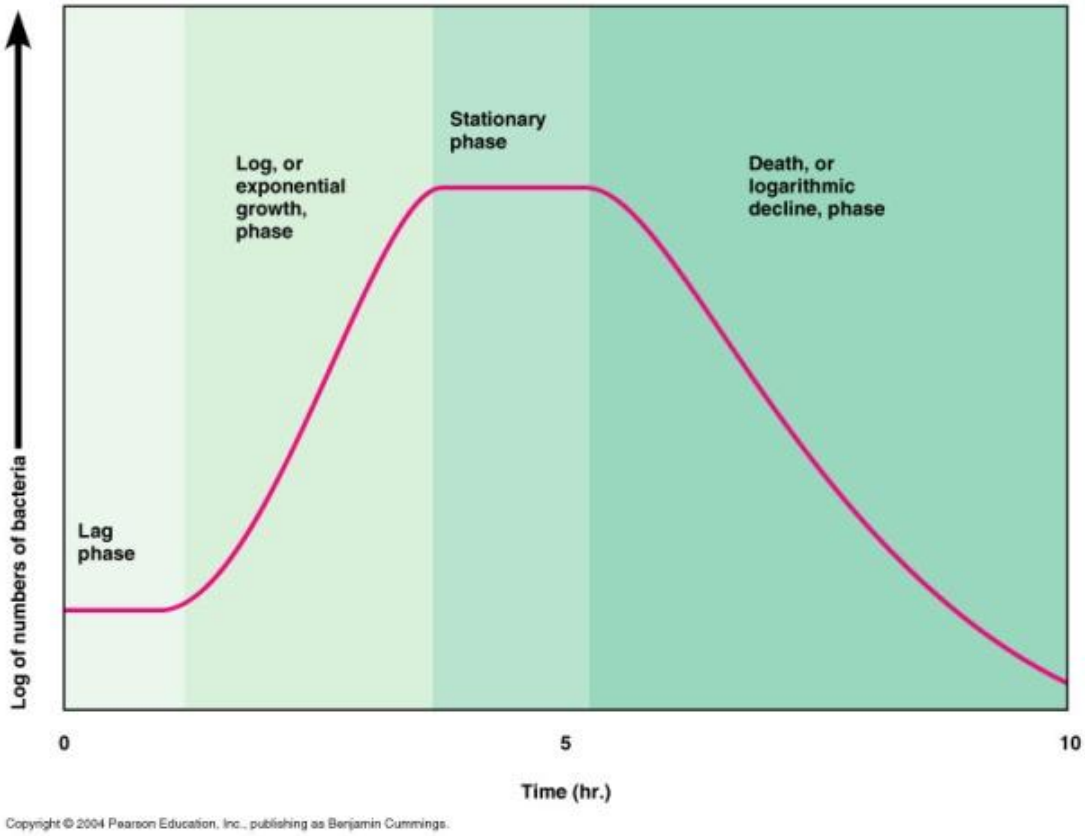
In order to get a better understanding of the cross contamination issue, first several testing was done on Medical Filters without antimicrobials. After that, we tested products with the selected antimicrobial ingredient.

### 2.4.2 Test results

The graph below shows the results of CFU measurements on Medical Filters without antimicrobials in 5 different periods of time.

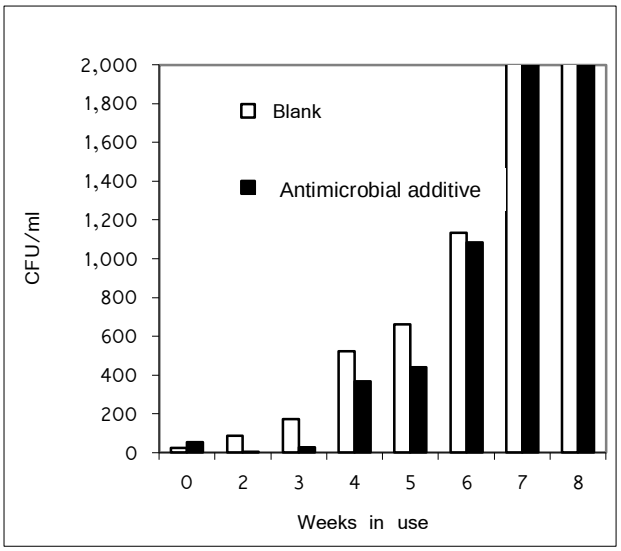



A clear trend is visible of increasing CFU/ml in the first 2 weeks which levels of at week 3 and then remains relatively constant. This trend may be explained by comparison to the stages in microbial growth which are shown below.




Generally in microbial growth first a lag phase occurs where growth is limited, followed by a logarithmic phase where the amount of bacteria doubles each generation time. Next a stationary phase is present where the total amount of living bacteria remains constant usually caused by a lack of nutrients. This is followed by a death phase where the amount of bacteria declines logarithmically, mostly due to accumulation of toxic substances.

The testing with Medical Filters with the selected antimicrobial showed a strong reduction of CFU values over the validated lifetime of 5 weeks of use. See the graph below.




### 2.4.3 Conclusion

Analysis of the effluent of the Shower Filters on pathogenic bacteria showed that these were not present in the measured CFU. Results are shown in the table below.

| | **4 weeks of use** | | | **8 weeks of use** | | |
|---|---|---|---|---|---|---|
| | **Filter 1** | **Filter 2** | **Filter 3** | **Filter 1** | **Filter 2** | **Filter 3** |
| **Coliforms (CFU/ml)** | <1 | <1 | <1 | <1 | <1 | <1 |
| ***E. Coli* (CFU/ml)** | <1 | <1 | <1 | <1 | <1 | <1 |
| ***Enterococcus* (CFU/ml)** | <1 | <1 | <1 | <1 | <1 | <1 |
| ***Pseudonomas aeruginosa* (CFU/ml)** | <1 | <1 | <1 | <1 | <1 | <1 |
| **MS 2 phages (CFU/ml)** | <1 | <1 | <1 | <1 | <1 | <1 |

Probably these are mostly harmless bacteria originating from the air which are able to grow in the moist environment after the membranes. However, in practical hospital situations, cross contamination via the air and/or persons cannot be excluded and can pose a threat to patients and staff, especially in the most critical wards.

The silver released from the plastic is accumulated in the 0.01 L compartment after the membranes but strongly diluted during use of the filter. While a concentration of between 10 and 100 ppb can be present during the standstill period, effluent values are always far below the WHO limit of 100 ppb.

The antimicrobial additive to the spray cap of the Medical Filters strongly reduces the cross contamination of the filters. The silver release however is far below toxicity levels and does not pose a threat to the users of the filters.

# 3. Chemical resistance

## *3.1 Test description*

To test the chemical resistance of the Medical Filters they were exposed to chlorine concentrations of 1200 ppm hypochlorite for 10 h and compared to blanks of unused filters and filters flushed for 10 h with tap water. Samples were evaluated both externally and internally for discolorations and defects, while furthermore membranes were evaluated by tensile strength measurements.

## *3.2 Test results*

Medical filters exposed to 1200 ppm hypochlorite were comparable the blanks, where no defects or discolorations were found (Fig. 1). Also tensile strength of the membranes was the same for both hypochlorite exposed and non exposed membranes.

Figure 1: Evaluation of shower filter for defects and discolorations

## *3.3 Conclusions*

Exposure to 1200 ppm hypochlorite for 10 h does not negatively influence the Medical Filters. Thus is can be concluded that Medical Filters are compatible with this chemical treatment.

# 4. Flowrate/pressure tests

## *4.1 Test description*

To evaluate the flow rate both Medical TapFilters and Medical ShowerFilter were flushed with tapwater at increasing pressure. Tests on the Medical ShowerFilter were performed with and without a 6 L/min flow restrictor, which is recommended for water saving purposed. Tests on the Medical TapFilter were performed with the obliged flow restrictor of 4 L/min.

## *4.2 Test results*

Results of the Medical ShowerFilter and Medical TapFilter are shown in Figure 2 and 3 respectively.

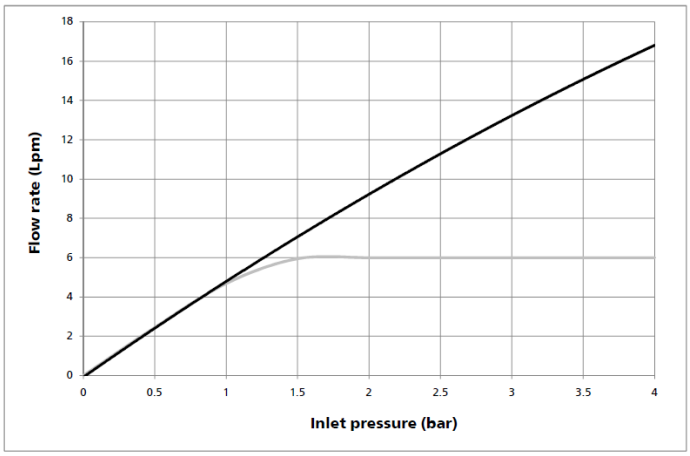



Figure 2: Flowrate-pressure curve of the Medical ShowerFilter with (gray) and without (black) a 6 L/min flow restrictor

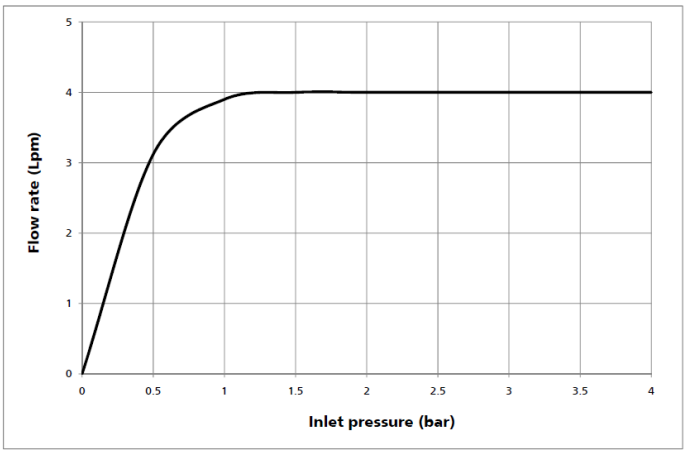



Figure 3: Flowrate-pressure curve of the Medical TapFilter with a 4 L/min flow restrictor

## *4.3 Conclusions*

Medical Filters show increasing flow rates with increasing pressure, where flowrate is leveled off at the desired level by use of a flow restrictor.

# 5. Appendices

## *5.1 Management Summary ASTM F838-05*

*Pseudomonas diminuta removal on Norit Filtrix Capfil Microfiltration Membrane - MF 02 M12 LE- sp*

**General**

Three microfiltration membrane cartridges, containing Norit Filtrix Capfil Microfiltration Membranes type MF 02 M12 LE sp, were tested according to ASTM International, Designation: F838-05"Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration".

The tests were performed in order to show that the cartridges can quantitatively retain large numbers of organisms ($10^7$ organisms per $cm^2$ of effective filtration area required by ASTM F 838-05).

**Used methods**

Testing was performed on three cartridges from October 20th, 2008 onward.
The tests were performed with bacteria *Pseudomonas diminuta* (ATCC 19146) under test conditions as specified in ASTM F 838-05.
The feed and filtrate samples taken from the challenge tests were analyzed by Vitens Laboratory, Leeuwarden, The Netherlands.
The analysis of the samples were conducted within 24 hours after the testing.
Detection and enumeration of the Pseudomonas *diminuta* was done according to ISO 9308-1.

**Test results**

| Filter | 1 | 2 | 3 | 1 | 2 | 3 |
|---|---|---|---|---|---|---|
| Filter load CFU/L | $8 \times 10^9$ | $8 \times 10^9$ | $6 \times 10^9$ | $8 \times 10^9$ | $8 \times 10^9$ | $6 \times 10^9$ |
| | after 5 L suspension filtrated | | | mixed sample from 5 L | | |
| Effluent CFU/L | <100 | <100 | <100 | <100 | <100 | <100 |
| Log reduction | >7.2 | >7.2 | >7.2 | >7.2 | >7.2 | >7.2 |

Note: The table above presents the results of the *Pseudomonas diminuta* challenge experiments, using the data from the analytical report of Vitens. The ASTM standard states a challenge of $10^7$ bacteria per $cm^2$ of effective filtration area (partition 4, page 1). As can be seen from the table all cartridges perform according to the standard.

## 5.2 Management Summary Klebsiella retention tests

**Management Summary**

*Klebsiella terrigena removal on Norit Filtrix Capfil Microfiltration Membrane - MF 02 M12 LE sp*

**General**

Three microfiltration membrane cartridges, containing Norit Filtrix Capfil Microfiltration Membranes type MF M12 LE sp, were tested under test conditions which are based on NSF Protocol P231 "Microbiological Water Purifiers" at Vitens Laboratories, Leeuwarden, The Netherlands.
The tests were performed in order to show that the cartridges have a bacterial retention level of =log 6 over a period of time, as required by NSF protocol P231.

**Used methods**

The testing was performed on three cartridges from 25th November 2008 onward. The tests were performed under test conditions which are based on NSF protocol P231 for microbiological water purifiers. The feed and filtrate samples taken from the challenge tests were analyzed by Vitens Laboratories, Leeuwarden, The Netherlands. Analysis of the samples was conducted within 24 hours after the testing. Detection and enumeration of the *Klebsiella terrigena* (ATCC 33257) was done according to ISO 9308.

**Test results**

The table below displays the results of the *Klebsiella terrigena* challenge experiments, using the data from the analytical report of Vitens. The samples were taken after 5 liters of filtrate.

| Filter | 1 | 2 | 3 |
|---|---|---|---|
| | Log reductions of effluent samples | | |
| Start of test | > 6 | > 6 | 3.8 |
| After 1 week | > 6.3 | > 6.3 | > 6.6 |
| After 2 weeks | > 6.9 | > 6.9 | > 6.9 |
| After 3 weeks | > 6.9 | > 6.9 | > 6.1 |
| After 4 weeks | > 7.5 | 7.2 | 7,4 |
| After 5 weeks | > 7.5 | > 7.5 | > 7.5 |

## Management Summary

### Conclusion

Filter 3 showed a performance below the standard right at the start of the test. As all other samples taken from this filter during the test showed a performance according to the requirements, the conclusion can be drawn that a contamination has occurred during either sampling or analysis.

The NSF Protocol P231 states a minimum reduction of log 6 on bacteria (Annex B, page 18). According to the NSF P 231 protocol, annex B, paragraph 3.5.3, ten percent of the samples pairs are allowed to fall below the requirement, while the geometric mean of all measured values must meet or exceed the requirements. In this test one sample is below log 6 reduction, however the geometrical mean of the complete series is 6.6, hence the data are acceptable.

The conclusion is that the bacterial retention requirements of the tests have been met.

## 5.3 Management Summary Legionella retention tests

**Management Summary**

*Legionella pneumophila removal on Norit Filtrix Capfil Microfiltration Membranes MF 02 M12 LE sp*

**General**

Three microfiltration membrane cartridges, containing Norit Filtrix Capfil Microfiltration Membranes type MF M12 LE sp, were tested under test conditions which are based on NSF Protocol P231 "Microbiological Water Purifiers" at Vitens Laboratories, Leeuwarden, The Netherlands. Tests were performed in order to show that the cartridges have a bacterial retention level of =log 6 required by NSF protocol P231 for the bacteria *Legionella pneumophila*.

**Used methods**

The testing was performed on three cartridges from 2nd July 2009 onward. Tests were performed under test conditions which are based on NSF protocol P231 for microbiological water purifiers. First the cartridges were flushed with 50 L filter sterilized water, followed by a challenge of 5 L water with a minimum concentration of $8 \times 10^8$ *Legionella pneumophila* (serotype 9) per liter. Effluent samples were taken at the end of the challenge followed by flush with 200 L filter sterilized water. The procedure was repeated 3 times per week over a period of 5 weeks. The feed and effluent samples taken from the challenge tests were analyzed by Vitens Laboratory, Leeuwarden, The Netherlands. Analysis of the samples was conducted within 24 hours after the testing. Detection and enumeration of the *Legionella pneumophila* (serotype 9) was done according to NEN 6265:2007.

## Management Summary

### Test results

The table below displays the results of the *Legionella pneumophila* challenge experiments, using the data from the analytical report of Vitens.

| Filter | 1 | 2 | 3 |
|---|---|---|---|
| | Log retention of effluent samples | | |
| Start of test | >6.8 | >6.8 | >6.8 |
| After 4 days | >7.0 | >7.0 | >7.0 |
| After 5 days | >7.5 | >7.5 | >7.5 |
| After 1 week | >7.6 | >7.6 | >7.6 |
| After 1 week and 4 days | >8.6 | >8.6 | >8.6 |
| After 1 week and 5 days | >7.5 | >7.5 | >7.5 |
| After 2 weeks | n.d. | n.d. | n.d. |
| After 2 week and 4 days | >8.5 | >8.5 | >8.5 |
| After 2 week and 5 days | >7.2 | >7.2 | >7.2 |
| After 3 weeks | >7.1 | >7.1 | >7.1 |
| After 3 week and 4 days | >7.0 | >7.0 | >7.0 |
| After 3 week and 5 days | >6.9 | >6.9 | >6.9 |
| After 4 weeks | >7.0 | >7.0 | >7.0 |
| After 4 week and 4 days | >7.1 | >7.1 | >7.1 |
| After 4 week and 5 days | >7.1 | >7.1 | >7.1 |
| After 5 weeks | >7.1 | >7.1 | >7.1 |

n.d.: no data due to an error in sample analysis

### Conclusion

The results of the retention tests are all above log 6.8, which is more than the minimum requirement of log 6 by NSF protocol P231.

It can be concluded that the Norit Filtrix Capfil Microfiltration Membranes meet the NSF retention requirements for *Legionella pneumophila*.

## 5.4 Management Summary Aspergillus fumigates retention tests

**Management Summary**

*Aspergillus fumigatus removal on Norit Filtrix Capfil Microfiltration Membranes*

**General**

Three microfiltration membrane cartridges, containing Norit Filtrix Capfil Microfiltration Membranes, were tested under test conditions which are based on NSF Protocol P231 "Microbiological Water Purifiers" at Vitens Laboratory, Leeuwarden, The Netherlands, an ISO 17025 accredited laboratory. Tests were performed in order to show that the cartridges are capable to achieve a minimum retention level of =log 4 for *Aspergillus fumigatus*.

**Used methods**

Tests were performed on three cartridges from 26th October 2009 under test conditions which are based on NSF protocol P231 for microbiological water purifiers. This protocol requires a minimum reduction of log 6 for bacteria and log 4 for viruses. There is no reduction level described for fungi in NSF protocol P231. Due to the larger size of fungi a minimum reduction level of log 4 was required in order to determine the capability of fungi reduction.

First the cartridges were flushed with 50 L filter sterilized water, followed by a challenge of 5 L water with a minimum concentration of 2 x $10^5$ *Aspergillus fumigatus* (water isolate V81-32) per liter. Effluent samples were taken at the end of the challenge followed by flush with 200 L filter sterilized water. The procedure was repeated 3 times per week over a period of 5 weeks. The feed and effluent samples taken from the challenge tests were analyzed by Vitens Laboratory, Leeuwarden, The Netherlands. Analysis of the samples were conducted within 24 hours after the challenge.

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**Test results**

The table below displays the results of the *Aspergillus fumigatus* challenge experiments, using the data from the analytical reports of Vitens Laboratory.

| | Log retention of *Aspergillus fumigatus* | | |
|---|---|---|---|
| Cartridge | 1 | 2 | 3 |
| Start of test | >4.1 | >4.1 | n.d. |
| After 1 day | >4.1 | >4.1 | n.d. |
| After 3 days | >4.0 | >4.0 | >4.0 |
| After 1 week | >4.3 | >4.3 | >4.3 |
| After 1 week and 1 day | >4.3 | >4.3 | >4.3 |
| After 1 week and 3 days | >4.2 | >4.2 | >4.2 |
| After 2 weeks | >4.3 | >4.3 | >4.3 |
| After 2 week and 1 day | >4.2 | >4.2 | >4.2 |
| After 2 week and 3 days | >4.1 | >4.1 | >4.1 |
| After 3 weeks | >4.4 | >4.4 | >4.4 |
| After 3 week and 1 day | >4.1 | >4.1 | >4.1 |
| After 3 week and 3 days | >4.4 | >4.4 | >4.4 |
| After 4 weeks | >4.5 | >4.5 | >4.5 |
| After 4 week and 1 day | >4.3 | >4.3 | >4.3 |
| After 4 week and 3 days | >4.0 | >4.0 | >4.0 |
| After 5 weeks | >4.3 | >4.3 | >4.3 |

n.d.: no data due to an error in sample acquisition

**Conclusion**

All samples show a retention performance above the goal of log 4. It can be concluded that over a period of at least 5 weeks Norit Filtrix Capfil Microfiltration meet the retention requirements for *Aspergillus fumigatus*.

## 5.5 Management Summary on Clinical Evaluation

**Management summary**

*Clinical evaluation of H2OK Medical Filters*

**Introduction**

During a routine check on 18 June 2009 *a* contamination with *Legionella* species was detected in the effluent of several showers in the Medical Spectrum Twente, location Ariënsplein, the Netherlands. After this detection all showers were replaced by Norit H2OK Medical Filters. This situation was considered suitable for clinical evaluation.

**Methods**

For this clinical evaluation five clinical wards equipped with the Medical Filters were chosen for further analysis. At each ward the effluent of two showers was analyzed for the presence of *Legionella* species one week after placement. Furthermore, at two wards two showers were weekly evaluated for a period of five weeks, the recommended replacement interval of the product. All samples were collected and analyzed for the presence of *Legionella* species by culture according to NEN 6265:2007, by Vitens Laboratory, Leeuwarden, the Netherlands, an ISO 17025 accredited laboratory. The detection level of the applied method was 100 *Legionella* cfu/ L.

**Results**

Results for 5 clinical wards after 1 week of use are shown in the table below.

| Ward | Shower number | Influent ( *Legionella* cfu/L) | Effluent (*Legionella* cfu/L) |
|---|---|---|---|
| **Coronary** | I | 8.600 | <100 |
| | II | 8.700 | <100 |
| **Urology** | I | 1.600 | <100 |
| | II | <100 | <100 |
| **Oncology** | I | 9.500 | <100 |
| | II | 4.900 | <100 |
| **Infectious disease** | I | <100 | <100 |
| | II | <100 | <100 |
| **Elderly nursing** | I | <100 | <100 |
| | II | <100 | <100 |

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Results during 5 weeks of use are shown in the table below.

| Ward | Week | Shower number | Influent (*Legionella* cfu/L) | Effluent (*Legionella* cfu/L) |
|---|---|---|---|---|
| **Coronary** | 0 | I | 8.400 | <100 |
| | | II | 36.000 | <100 |
| | 1 | I | 20.000 | <100 |
| | | II | 35.500 | <100 |
| | 2 | I | 13.000 | <100 |
| | | II | 22.500 | <100 |
| | 3 | I | 42.000 | <100 |
| | | II | 11.000 | <100 |
| | 4 | I | 8.000 | <100 |
| | | II | 5.300 | <100 |
| | 5 | I | 15.500 | <100 |
| | | II | 7.700 | <100 |
| | | | | |
| **Urology** | 0 | I | 100 | <100 |
| | | II | 2.900 | <100 |
| | 1 | I | 1.600 | <100 |
| | | II | 3.900 | <100 |
| | 2 | I | 7.300 | <100 |
| | | II | 1.200 | <100 |
| | 3 | I | 350 | <100 |
| | | II | 6.700 | <100 |
| | 4 | I | <100 | <100 |
| | | II | 1.400 | <100 |
| | 5 | I | 100 | <100 |
| | | II | 600 | <100 |

**Conclusion**

Fifty percent of the influent samples of 5 wards was contaminated with *Legionella* species, while in the effluent samples no *Legionella* species were detected after 1 week usage. Furthermore, at two wards for a five week period 95 % of influent samples were contaminated while again no *Legionella* species were detected in the effluent. Effective *Legionella* species retention by the Norit H2OK Medical Filters was shown in this clinical study.

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