



# Applied Industrial Microbiology, Inc.

## TEST REPORT

2321 South Melrose Drive, Vista, CA 92081  
Phone:(760)-295-0430 Fax:(760)-734-4454  
Email: [Info@Aimvistalab.com](mailto:Info@Aimvistalab.com)  
[WWW.AIMVISTALAB.COM](http://WWW.AIMVISTALAB.COM)

**Client Information:** Chris Burke  
Safe Water Products

**Report Date:** 03/20/2017  
**Matrix:** Filter  
**Collected:** 01/13/2017 @  
**Sampled By:** JD Moore

**Reference Number:** 1703406

**Collection Address:**  
**Description:** Point Of Use (POU) Legionella Filter

**Lab ID:** 1703406-001

**Received:** 01/13/2017 @ 13:55

**Sample#:**

**Comment:** Safe Water Products HFM Filter (HFM = Hollow Fiber Membrane).  
Amendment report for sample received 01/13/2017

**Date Completed:** 03/20/2017

### Filter efficacy by bacterial log reduction

| <u>Test Parameter</u>            | <u>Result</u> | <u>Unit</u> | <u>RL</u> | <u>MCL</u> | <u>Method</u> | <u>Analyzed</u>  | <u>Analyst</u> |
|----------------------------------|---------------|-------------|-----------|------------|---------------|------------------|----------------|
| Legionella sp. After Filtration  | <1            | CFU/ml      | 1         | 0          | CDC ELITE     | 02/22/2017 10:30 | HD             |
| Legionella sp. Before Filtration | 30,000,000    | CFU/ml      | 1         | 0          | CDC ELITE     | 02/22/2017 10:30 | HD             |

Approval:

Xzayla Zabiti  
QA Officer  
Xzayla Zabiti

[Signature]  
Laboratory Director  
Hojabr Dezfulian, Ph.D.

RL = Reporting Limit

MCL = Maximum Contaminant Level

N/A = Not Applicable

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Unless otherwise noted, sample(s) were received in acceptable condition. The results in this report relate only to the portion of the sample(s) tested. This report shall not be reproduced, except in full, without approval of the laboratory. This document contains confidential commercial information pursuant to 5U.S.C. § 552(b)(4). ©2014 AIM.

DISCLAIMER: Applied Industrial Microbiology laboratory did not collect the analyzed sample(s) and thus accepts no liability with regard to their collection and/or maintenance. Our laboratory relies on the client for all sample information prior to delivery at our address.



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03/20/2017 (Revised)

The goal of this study was to prove that the Safe Water Products HFM Filter (HFM = Hollow Fiber Membrane) (Sample ID number 1703406-001) can reduce the load of *Legionella pneumophila* by 7 logs.

Serial dilutions of *Legionella pneumophila* ATCC 33152 were made from McFarland standard no1. with an approximate cell count density of  $3 \times 10^8$  (Figure. 1).

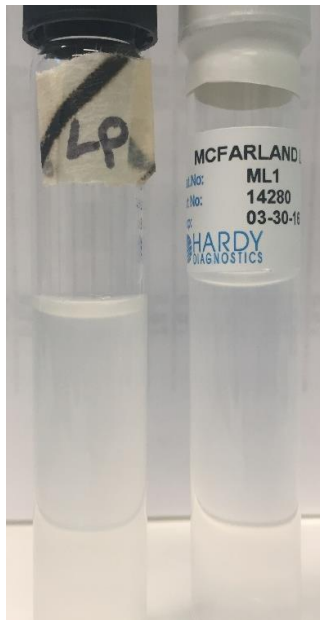


Figure. 1: Preparation of McFarland Standard

Serial dilution tubes, of  $10^{-7}$  and  $10^{-8}$  were plated on Buffered Charcoal Yeast Extract (BCYE) agar.



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Three colonies grew on the plate of  $10^{-7}$  and there was no colony growth for the plate of  $10^{-8}$  (Figure.2).

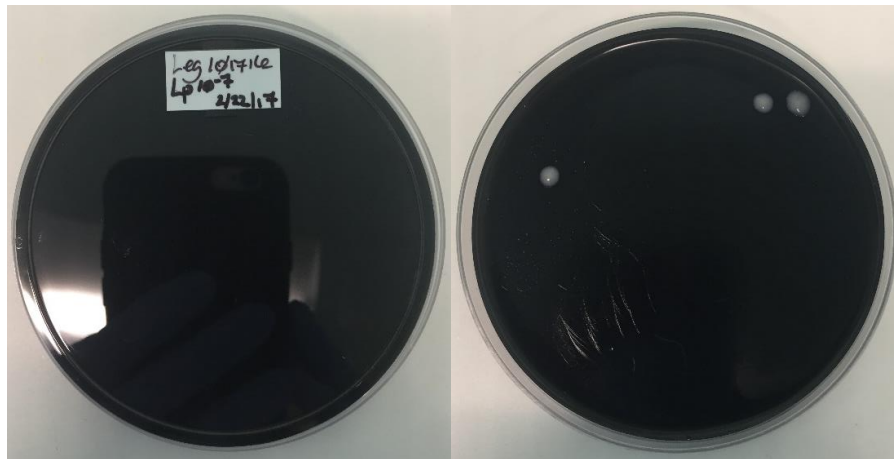


Figure 2.: Verification of the concentration of bacteria.

Therefore, the approximate number of bacteria in the inoculum was  $3 \times 10^7$  CFU/mL.

One liter of distilled water was sterilized and inoculated with 1 mL of the inoculum. This sample was filtered through the HFM Filter (Figure. 3).



Figure. 3: HFM Filter assembly



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The filtrated water was then processed per “Procedures for the Recovery of *Legionella* from the Environment” from National Center for Infectious Diseases, Division of Bacterial and Mycotic Diseases, Respiratory Disease Laboratory Section, January 2005.

In summary, one liter of the filtrate was filter concentrated by pouring the sample into a sterile 47-mm filter funnel assembly containing a 0.2  $\mu\text{m}$  polycarbonate filter. The filter was removed aseptically from the holder with sterile filter forceps, folded to the outside, and placed into a sterile, 50-ml centrifuge tube containing 5ml of sterile water. The centrifuge tube was then vortexed for one (1) minute to free bacteria from the filter.

After vortexing of the filter, one BCYE plate was inoculated with 1 mL of the suspension, spread with a sterile disposable plastic rod and incubated at 35°C in a candle jar in a humidified incubator with an atmosphere of approximately 2.5% CO<sub>2</sub> in air. The remainder of the sample was stored at 4°C.

After seven days, the plate was examined for the growth of the *Legionella pneumophila*. Thirteen (13) colonies were detected on the plate (Figure. 4).

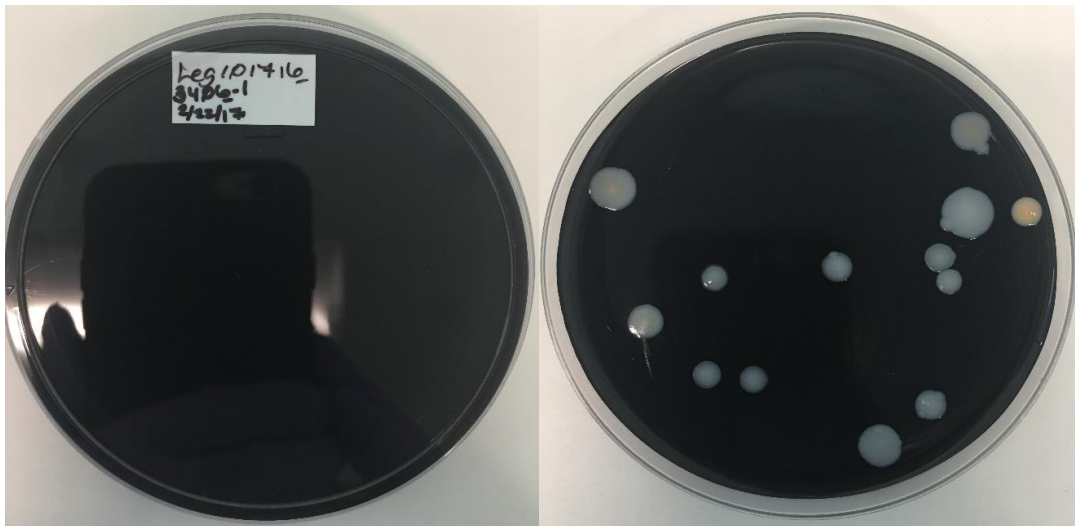


Figure. 4: Concentrated filtrate.



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The concentration factor is 200X, therefore the filtrate contained  $2.6 \times 10^3$  CFU/mL of bacteria. In house PCR, as well as CDC ELITE protocol, were applied to each colony. None of the colonies were *Legionella* (Figure.5).

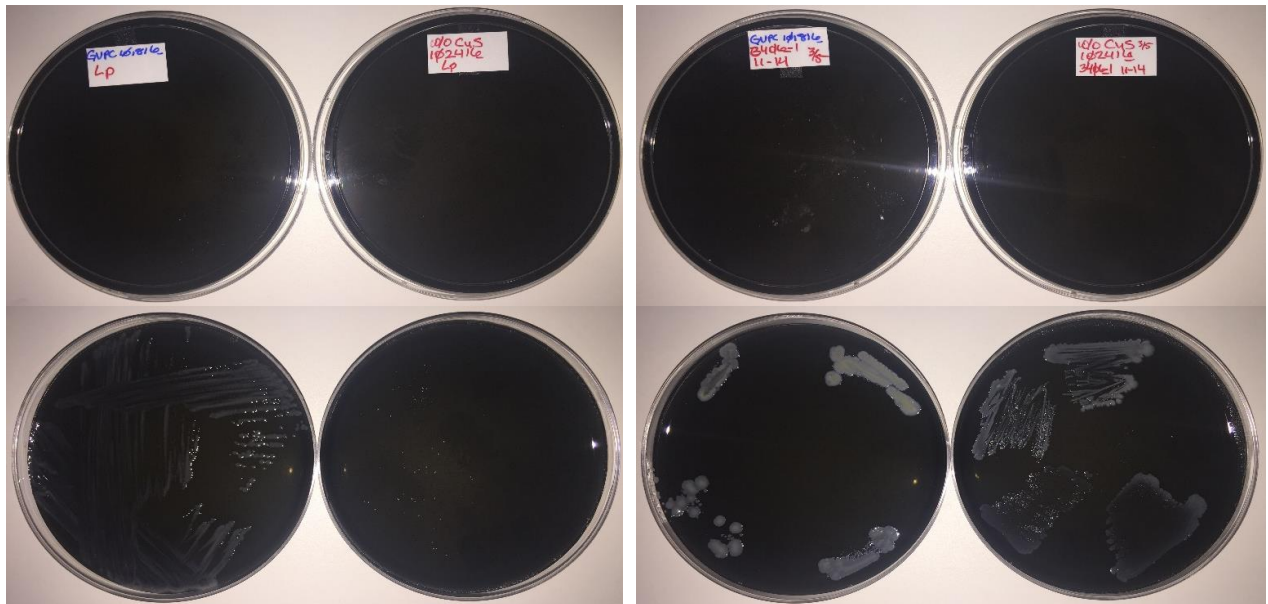


Figure.5: On the left growth of *Legionella pneumophila* on Buffered Charcoal Yeast Extract Agar with Glycine, Vancomycin, Polymyxin, and Cycloheximide (GVPC-BCYE) and no growth on BCYE without L-cysteine. On the right the growth of filtrate bacteria on both agars. This indicates these bacteria are not *Legionella pneumophila*.

This indicates that the Safe Water Products HFM Filter can reduce the number of *Legionella pneumophila* by at least 7 logs.

Best Regards

Xzayla Zabiti

Xzayla Zabiti  
Vice President of Quality Assurance