



# Certificate of Analysis

## SUMMIT LABORATORY, LLC

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**Client:** Ola Filter Corporation  
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**Report Date:** June 28, 2022

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**Summit Laboratory Job #:** 2206613 (page 1 of 2)

**Analysis Requested:** Microbiological challenge testing with *Escherichia coli* (ATCC 8739, AOAC 991.14, SM9223B)

Following is the results report for the "OLA water filter" micro challenge project:

### Summary

The Ola water filter was challenge tested for its ability to remove the microorganism *Escherichia coli* (*E. coli*). *E. coli* was purposely added to the test water at a concentration of  $10^7$ . The tested Ola water filter met the target reduction of 6 log units, or 99.9999% for all three test runs.

### Introduction

Filtration is "a pressure or gravity driven separation process in which particulate matter larger than 1um is collected by an engineered barrier primarily through a size exclusion mechanism and which has a measureable removal efficiency of a target organism that can be verified through the application of a direct integrity test" (40 CFR 141.2). The water filter underwent challenge testing with a specific microorganism to determine if the filter performed as a barrier.

An Ola water filter was challenge tested in triplicate. The filter was initially conditioned with a 5% chlorine solution and sterile tested to be free of any *E. coli* and background bacteria. The challenge microorganism (*E. coli*) was mixed with the test water to obtain at least a  $10^6$  cells per 100 mL concentration and was forced through the Ola water filter. Three 100mL of filtrate samples were collected in a sterile EPA coliform water container after the 100mL passed through the OLA water filter and analyzed for *E. coli* microbial growth using both the Colilert technique following the Standard Methods 9223B and the AOAC 991.14 petrifilm method.

### Methods

Stock cultures of *E. coli* (ATCC 8739, Lot: 483-1122-2, Exp: 3-31-2023) were streaked onto Trypticase soy agar (TSA, Lot: US113375B, Exp: 7-31-2025) plates and incubated at 35°C for 24 hours. Pure culture colonies were selected from the plate. Ten pure colonies were inoculated into 500mL of reagent water (3M Butterfield's buffer water, Lot: 33KELL, Exp: 2024-05-03). The test water was inoculated to determine that the obtained final density was  $1.8 \times 10^7$  cells/100 mL.

### Microbiological analysis

Standard Methods 9223B and AOAC 991.15 were followed. The following procedures were executed in triplicate to obtain triplicate results. Each of the three independent 100mL *E. coli* samples were homogenized thoroughly and poured into the sterile squeeze bag, then connected to the hose that was connected to the Ola water filter. By both gravity and hand pressure (squeezing the bag) the 100mL of inoculated water samples were collected after going through the Ola water filter. Ola filtered water was collected into a sterile 100mL EPA coliform container (Lot: 1809289898, Exp: 9-28-2023) and tested following the Colilert method and the AOAC 991.14 petrifilm method. A 1mL aliquot of the filtered water was serial diluted and inoculated onto the 3M *E. coli* petrifilm, dilution samples tested were 1:1 (direct test sample),  $10^3$ , and  $10^5$ . A 1mL aliquot of the three diluted samples inoculated onto an *E. coli* petrifilm and incubated at 35°C for 48 hours. After the petrifilm aliquots were taken out of the sample the remaining test sample was enriched directly with approximately 3 grams of Colilert media (individual packets for direct test). After the Colilert media is dissolved completely in the test sample the test sample is emptied into a sterile Quanti-tray (Lot: CU022J, Exp: 3-4-2025), ran through the Quanti-tray sealer then incubated at 35°C incubator for at least 24 hours.

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## Results

All trials had similar outcomes of zero cfu/mL and zero cfu/100mL (Table 1). All trials attained 6 log unit reduction or higher.

Table 1: Challenge Ola water filter test trials. Filtrate collected at 100 milliliters underwent microbiological MPN and petrifilm, values expressed as colon forming units per 1 milliliters and 100 milliliters.

Trial	Organism	Initial seed	1mL sample results:	100mL sample results:
		(cfu/mL)	(petrifilm)	(Colilert)
			(cfu/mL)	(cfu/100mL)
Control (challenge water)	<i>Escherichia coli</i>	180,000 (1.8 x 10 <sup>5</sup> )	180,000 (1.8 x 10 <sup>5</sup> )	18,000,000 (1.8 x 10 <sup>7</sup> )
Blank before testing (squeeze bag)	None	NA	<1	<1
1 (Ola water filter)	<i>Escherichia coli</i>	180,000 (1.8 x 10 <sup>5</sup> )	<1	<1
2 (Ola water filter)	<i>Escherichia coli</i>	180,000 (1.8 x 10 <sup>5</sup> )	<1	<1
3 (Ola water filter)	<i>Escherichia coli</i>	180,000 (1.8 x 10 <sup>5</sup> )	<1	<1

## Discussion

All three trials of the Ola water filter tested showed a 6 log unit or greater reduction of the test organism, indicating the filter successfully removes the organism from the challenge water.

## References

Microbiological analyses are in accordance with the Standard Methods for the Examination of Water and Wastewater, the 19<sup>th</sup> Edition, 1995, or approved U.S. *Environmental Protection Agency* (EPA) methodology, the Manual of Environmental Microbiology, 2<sup>nd</sup> Edition, 2002, Association of Official Agricultural Chemists (AOAC International), Microbiologics Inc., and/or current officially approved methodologies. Results reported are provided "as is" and relate only to the samples tested.

Report approved by:

Michael Snarski  
 Manager