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Survivor Filter Report No.:102092758COL-003

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Non-Standardized Test Report For:

Survivor Filter, a division of Zakaib Holdings Limited

Model: Survivor Filter <sup>™</sup> Triple Absolute Filtration to 0.05 Microns, and Survivor Filter <sup>™</sup> PRO 0.01 Micron Water Purifier Project No. G102092758

## **REVISION TABLE**

Date / Project Number	Engineer / F	Reviewer	Pages	Comments
06/20/2018 – G102092758	N. Unger L. Moomaw	NTU 1911	7	Corrected percentage from 99.9% to 99.999% reflecting 5 log reduction per client request.

Signature on File
N. Unger
Engineer

Signature on File
J. Senediak
Reviewer

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# **Objective**

The primary objective of this evaluation is to provide test data showing the amount of bacteria, virus, removed from a water matrix by the Survivor Filter and Survivor Filter Pro.

### **Overview**

A challenge suspension of known concentration will be prepared and introduced into the system. The suspension will be run through the filter and then analyzed for remaining concentrations.

Separate and new filters are to be used for the bacteria, virus, and heavy metals.

#### Detection will be as follows:

- I. Bacteria
  - a. Collection and enumeration technique derived from the Standard Total Coliform Membrane
     Filter Procedure in accordance with APHA's Standard Methods for the Examination of Water and
     Wastewater. This procedure is commonly used for NSF/ANSI certification standards for food
     equipment.
- II. Virus
  - a. Collection and enumeration technique derived from the Standard Total Coliform Membrane Filter Procedure in accordance with APHA's Standard Methods for the Examination of Water and Wastewater. This procedure is commonly used for NSF/ANSI certification standards for food equipment.
- III. Heavy Metals
  - a. The Elemental analysis of the Lead, Mercury and Cadmium will follow the IEC 62321 Elemental analysis procedure. This procedure is commonly used for RoHS analysis of various materials.

## **Procedural**

- I. Bacterial Analysis
  - a. Challenge Suspension Preparation
    - 1. E. coli, (ATCC 11229) and S. aureus (ATCC 6538) bacteria obtained from ATCC
    - 2. Stock cultures rehydrated with Tryptic Soy Broth (TSB) and incubated at  $36 \pm 1$  °C (97  $\pm 1$  °F) for approximately 24 hours
    - 3. The suspension will be diluted with phosphate buffer solution (PBS) to yield a concentration of 1 to  $5x10^6$  cfu/mL of each organism
  - b. Test
    - 1. 100 mL of the challenge suspension is filtered through the Survivor unit
    - 2. Samples will be serial diluted and will be plated on both MacConkey's agar and Mannitol Salts agar, inverted, and incubated at  $36 \pm 1$  °C (97  $\pm 1$  °F) for 24 h
      - i. Serial dilution plating will be performed in duplicate
    - 3. Plates are to be enumerated after the incubation period and results expressed as the number of CFU/mL
      - i. Only plates within the range of 20-300 cfu will be reported



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- 4. Control Samples to be performed as follows:
  - i. Negative control: PBS will be filtered through unit and enumerated
  - ii. Positive control: Serial dilution of the bacterial suspension will be performed using PBS and aseptically processed using the membrane filter technique
- 5. Steps (b)1-3 are repeated for Survivor Filter Pro Model

### II. Viral Analysis

- a. Challenge Suspension Preparation
  - 1. Phi X174 (#124425) to be obtained from Carolina Biosciences
  - 2. Sample to be enumerated via serial dilution to confirm concentration
  - 3. A dilution of the stock suspension will be made so that the density of viral particles is approximately 1 to 5x10<sup>6</sup> plaque forming units (PFU) per mL
- b. Test Procedure
  - 1. 100 mL of the challenge suspension is filtered through the Survivor unit
  - 2. Samples will be serial diluted will be plated on nutrient agar utilizing the agar overlay technique, inverted, and incubated at  $36 \pm 1$  °C (97  $\pm 1$  °F) for 24 h
    - i. Serial dilution plating will be performed in duplicate
  - Plates are to be enumerated after the incubation period and results expressed as the number of PFU/mL
    - i. Only plates within the range of 20-300 PFU will be reported
  - 4. Control Samples to be performed as follows:
    - i. Negative control: PBS will be filtered through unit and enumerated
    - Positive control: Serial dilution of the viral suspension will be performed using PBS and aseptically processed using the membrane filter technique
  - 5. Steps (b)1-3 are repeated for Survivor Filter Pro Model

## **Parameters**

A digital hygrometer will be used to ensure the temperature range stayed within that specified in the procedure above.



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# **Hypothesis**

The hypothesis for the microbiological portion is that the percent reduction is measurable outside the tolerance of the method, which is 8%. This tolerance is based on the within laboratory precision of the standard plate count test that accounts for a within-analyst variation of 8% (AATCC 100-2012).

## **Sample Acquisition**

Sample will be submitted by the client for evaluation.

Sample #	Description	Serial #	Model	Date	Condition
COL1508141358 -001	Survivor Filter	X000M99LWN	Survivor Filter TM Model L600	7/20/2015	New
_					
Sample #	Description	Serial #	Model	Date	Condition

## **Equipment List**

Item	Equipment Type	Intertek Asset No.	Calibration Due
1	Micropipette	CE 1141	03/11/16
2	Thermofisher Heracell Incubator	CE 2381	For Reference Only
3	Balance	CE 1182	11/3/15
4	Autoclave	CE 2376	Verify before use
5	Graduated Cylinder	CE 2264	Initial Calibration Only
7	Centrifuge	CE 2382	For Reference Only
8	Filtration System	CE 2031	Initial Calibration Only
9	Refrigerator	CE 1157	05/05/16
10	10-100 μL Pipette	CE2315	12/01/15
11	100-1000 μL Pipette	CE2234	03/30/16
12	1-5 ml Pipette	CE2228	03/30/16
13	Analytical Balance	CE2235	09/16/15
14	ICP	CE2100	Verify Before Use



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Testing to be conducted at: Intertek Testing Laboratory

1717 Arlingate Lane Columbus, OH 43228

## **Raw Microbiological Test Data**

Test Parameter		Result <sup>1</sup>	Units
Organism	Bacterial Species	Escherichia coli	
	ATCC No.	11229	
	Challenge Concentration	4.45 x 10 <sup>6</sup>	CFU/mL
Survivor Filter <sup>™</sup> Triple	Serial Dilution Replicate 1	<1	CFU/mL
Absolute Filtration to	Serial Dilution Replicate 2	<1	CFU/mL
0.05 Microns	Average	<1	CFU/mL
Survivor Filter <sup>™</sup> PRO	Serial Dilution Replicate 1	<1	CFU/mL
0.01 Micron Water	Serial Dilution Replicate 2	<1	CFU/mL
Purifier	Average	<1	CFU/mL

<sup>&</sup>lt;sup>1</sup>Results imported from Intertek Report Number 102092758COL-002

Test Parameter		Result <sup>1</sup>	Units
Organism	Bacterial Species	Staphylococcus aureus	
	ATCC No.	ATCC 6538	
	Challenge Concentration	4.96 x 10 <sup>6</sup>	CFU/mL
Survivor Filter <sup>™</sup> Triple	Serial Dilution Replicate 1	<1	CFU/mL
Absolute Filtration to	Serial Dilution Replicate 2	<1	CFU/mL
0.05 Microns	Average	<1	CFU/mL
Survivor Filter <sup>™</sup> PRO	Serial Dilution Replicate 1	<1	CFU/mL
0.01 Micron Water	Serial Dilution Replicate 2	<1	CFU/mL
Purifier	Average	<1	CFU/mL

<sup>&</sup>lt;sup>1</sup>Results imported from Intertek Report Number 102092758COL-002

Test Parameter		Result <sup>1</sup>	Units
Organism	Viral Species	Phi X174	
	ATCC No.	13706-B1	
	Challenge Concentration	5.0 x 10 <sup>6</sup>	PFU/mL
Survivor Filter <sup>™</sup> Triple	Serial Dilution Replicate 1	740	PFU/mL
Absolute Filtration to	Serial Dilution Replicate 2	810	PFU/mL
0.05 Microns	Average	775	PFU/mL
Survivor Filter <sup>™</sup> PRO	Serial Dilution Replicate 1	<1	PFU/mL
0.01 Micron Water	Serial Dilution Replicate 2	<1	PFU/mL
Purifier	Average	<1	PFU/mL

<sup>&</sup>lt;sup>1</sup>Results imported from Intertek Report Number 102092758COL-002



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## **Percent Reduction Calculations**

Percent reduction calculated as follows-

Percent Reduction =  $(A-B) \times 100$ 

Where:

A is the number organism concentration before running through the filter. B is the number organism concentration after running through the filter.

## **Percent Reduction Values - Microbiological**

	Bacterial Challenge <sup>1</sup>		Viral Challenge <sup>1</sup>
Unit Type	E.coli	S.aureus	Phi-X174
Survivor Filter TM Triple Absolute Filtration to 0.05 Microns	>99.999%	>99.999%	99.999%

<sup>&</sup>lt;sup>1</sup>Results imported from Intertek Report Number 102092758COL-002

	Bacterial Challenge <sup>1</sup>		Viral Challenge <sup>1</sup>
Unit Type	E.coli	S.aureus	Phi-X174
Survivor Filter <sup>™</sup> PRO 0.01 Micron Water Purifier	>99.999%	>99.999%	>99.999%

<sup>&</sup>lt;sup>1</sup>Results imported from Intertek Report Number 102092758COL-002





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## **Conclusion**

The microbiological hypothesis has been accepted since the plate count values were well outside the 8% measurement of uncertainty of the microbiological test method.