



# Stability of *Aeromonas hydrophila* in Tap Water With Chlorine Residual

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## Abstract

Safe water is essential to protect public health. Therefore, water supplies in the US are typically treated to remove microbial contamination and a residual is applied to protect the water during distribution. The primary purpose of this project is to test the stability of *Aeromonas hydrophila* in the presence of a typical chlorine residual. A series of time points were examined to evaluate the survival of *A. hydrophila* and its viability at each time point was measured via membrane filtration (MF) on both mixed cellulose ester and nitrocellulose 0.45µm filters. Results showed that the chlorine residual efficiently kills *Aeromonas*, however when the residual was neutralized *A. hydrophila* survived for the period tested.

## Introduction

- Waterborne diseases are caused by pathogenic microorganisms such as bacteria, viruses or protozoan parasites living in water. These microbes may be present within the water naturally or may be excreted in feces of infected persons.

- People drinking or coming into contact with untreated water (i.e., swimming, bathing) are potentially at risk for infection.

- A. hydrophila* is a gram-negative bacterium that is commonly present in freshwater and may cause gastroenteritis in humans.

- For the public health protection, chlorine residual is applied during the last stage of water treatment.

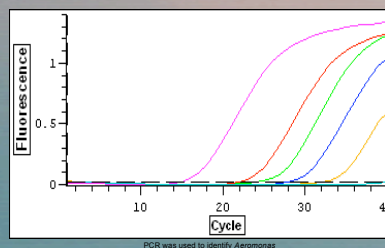
- Occasionally, due to pipes leakage, contamination of the distribution system occurs.

## Objective

- To evaluate the Stability of *A. hydrophila* in the presence of chlorine residual.

- To evaluate the potential impact of different filter composition on membrane filter recovery.

- It is hypothesized that the organism's die-off rate will gradually increase with the prolongation of its exposure to the tap water with the chlorine residual.



## Methodology

### Organism and Media

- A. hydrophila* (ATCC # 7966)
- Ampicillin-Dextrin Agar with Vancomycin (ADA-V)

### Membrane Filtration

- EPA Method 1605
- Two 0.45µm filters mixed cellulose ester (Pall) and nitrocellulose (Millipore type HA)

### Other Quantification Methods

- Spot titer
- qPCR (Sybr Green; primers from Kincombe, et al., 1999)<sup>1</sup>

### Experimental Design

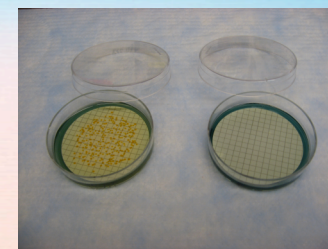
- 1L volumes of tap water (with residual and neutralized) seeded with a 1ml of *Aeromonas*.
- Aeromonas* concentration was observed over time (0, 15, 30, 60, and 180 minutes) by MF (1ml, 10ml, and 100ml) in duplicates.
- samples were neutralized with sodium thiosulfate prior to filtration.



Spot titered *Aeromonas*

## Results

- Aeromonas* is stable in dechlorinated water for at least 3hrs.
- In the presence of a chlorine residual (0.98 mg/L), *Aeromonas* is rapidly inactivated.
- There is no a detectable difference in the bacterial recoveries between the two types of membrane filters.



(R-L) The results of chlorinated vs. dechlorinated water

## Discussion

- Results indicate that *Aeromonas* can not survive in the presence of chlorine residual. This is consistent with an earlier study's outcome<sup>2</sup>.
- Qualitatively, the outcome of the two membrane filtration's experiment is consistent with earlier study<sup>3</sup>.
- We can prolong the experiment's time to 24hrs and study the stability of the organism in such, relatively, long period.
- Aeromonas* is stable for at least 3hrs in dechlorinated water but additional time points should be evaluated.

## Acknowledgments

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## References

- Cesar Isigdi Bin Kingombe, Geert Huys, Mauro Tonolla, M. John Albert, Jean Swings, Farraele Peduzzi, and Thomas Jenmi. 1999. 'PCR Detection, Characterization, and Distribution of Virulence Genes in aeromonas spp.' Applied and Environmental Microbiology, P. 5299-5302
- Tarakoli A, Yazdani R, Shahmansouri MR, Isfahani BN. 2002. 'Chlorine residual efficiency in inactivating bacteria from secondary contamination in Isfahan' East Mediterr Health J. (3):425-34.
- Green BL, Clausen E, Litsky W. 1975. 'Comparison of the new millipore HC with conventional membrane filters for enumeration of fecal coliform bacteria' Appl. Microbiol. (4):697-93.