Water Pouch Sterility: A 10 Year Study

Beth Skiles, BS, RVT, rLATG[1]; Debra L. Hickman, DVM, MS, DACLAM[2]; Nancy Johnston, DVM, MS, DACLAM[1]
[1]Laboratory Animal Resource Center, Indiana University School of Medicine, Indianapolis, Indiana
[2]AAALAC International

ABSTRACT

The in-house produced, commercially available water pouch system provides ultra-filtered, chlorinated sterile water to our rodent population. We wanted to perform testing to determine the possible shelf life of these water pouches. Pouches were produced in October 2015 using a dilute chlorine solution added to ultra-filtered water. Pouches were saved in standard storage containers in a typical storage room environment. We swabbed pouches at regular intervals for ten years. We used a commercially available ATP detection system to swab the inside surface of the pouch and water. The data demonstrates that in-house made water pouches remain sterile for at least 10 years.

INTRODUCTION

An important aspect of high quality mouse husbandry is providing high quality water. The Guide states that “animals should have access to potable, uncontaminated drinking water…”(1). Most researchers require sterility in all aspects of the microenvironment. The water provided to laboratory mice needs to be as close to sterile as possible since bacterial contamination may cause systemic disease in immunocompromised animals, or unwanted or unknown biologic variables. All watering systems for laboratory rodents have potential advantages and disadvantages. Water bottles are often sterilized by autoclave. However, the sterility of autoclaved water bottles and sipper tubes is not indefinite; the recommended shelf life of a case of wrapped water bottles to be no more than 22 days (2). Automatic watering systems also require frequent cleaning to prevent the formation of bacterial biofilm (3).

The water pouch system is an alternative water supply system. The water inside each pouch is sterile when produced. Our facility wanted to determine the possible shelf-life for these pouches. We tested pouches at many time points in the first year, every 6 months from 1 to 5 years, and yearly from 6 to 10 years.

METHODS

In October of 2015, water pouches were produced in-house using a commercially available system that provides ultra-filtered, chlorinated sterile water (5-10ppm). Water pouches were collected from the beginning, middle, and end of the run of 200 pouches. Total chlorine was measured daily for 7 days. Total chlorine was 7.7 ppm on day 1. Total chlorine was 3.4 ppm at day 7, as the chlorine dissipated over time as expected. The water pouches were stored in standard storage containers in a storage environment room where the temperature was 70 ° F +/- 2° .

At each time point, the external surface of the pouch was sterilized with an alcohol wipe. Water inside the pouches was swabbed; one water pouch from beginning, middle, and end of the run on day 1, weeks 1 and 2, months 1, 2, 4, 6, 12,18, 24, 30, and 36. One pouch was swabbed at 42, 48, 54, and 60 months, and years 6 through 10.

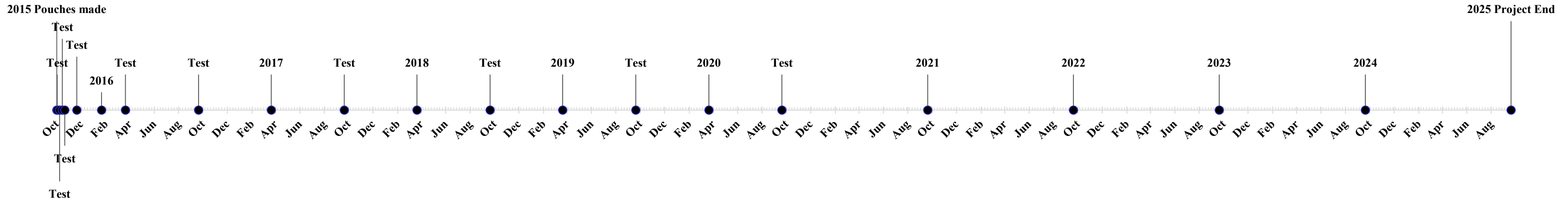
Each water pouch was swabbed using a sterile commercially available ATP swab. The corner of the water pouch was cut off using scissors that had been wiped with a sterile alcohol wipe. Care was taken not to touch the outside of the pouch while inserting the ATP swab into the water pouch. The inside of the pouch was swabbed including the sides of the pouch in case there were biofilm present on the sides of the water pouch.

Once the water pouch was swabbed using the ATP swab the swab was tested using the commercially available ATP detection system that uses ATP bioluminescence to detect ATP. The results of the test were recorded for each water pouch.

New Pouch – Not Taut

6 yo Pouch – Taut

RESULTS



All swab readings were 0 Relative Light Units, indicating that no organic material was detected in the water or on the internal surface of the water pouch. Data indicate that the water was sterile.

DISCUSSION

Our data indicate that in-house produced water pouches remain sterile for 10 years under typical storage conditions. We did notice that several pouches became more taut compared to new pouches at year 6. However, this change in the tightness of the pouch did not result any change in ATP bioluminescence. The plastic film is sealed during the sterile production process, protecting the water from possible contamination. This water production method has proven to provide sterile water in easily stored pouches with a very long shelf life. Based on this data, our facility maintains a reserve stock of water pouches for emergency use that is rotated every 6 months.

REFERENCES

1. **Guide for the Care and Use of Laboratory Animals, 8th edition. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Washington (DC): National Academies Press (US); 2011.**

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3. **Sanitization of an automatic reverse-osmosis watering system: removal of a clinically significant biofilm. Molk DM, Karr-May CL, Trang ED, Sanders GE. J Am Assoc Lab Anim Sci. 2013 Mar;52(2):197-205.**