

"Just Let it Flow"

A Closer Look at the Quality and Sterility of Standard NSG Mouse Drinking Water

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Abstract

Everyone knows the saying “water is essential to life.” However, does one type of water excel over the rest? Husbandry practices for immunodeficient strains such as NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) mice are at a higher standard to ensure animal welfare and consistent research results. In addition to getting weekly cage changes, NSG mice commonly receive acidified or autoclaved acidified water bottles. Because NSG mice are to be handled and cared for with delicacy in order to protect their health and the research results, we wanted to compare pH levels and microbial growth over the course of 1 week in the two most common types of water delivered to immunodeficient mice in our facility. The purpose of this study was to discover whether or not the pH level was affected by autoclaving and/or time, and if microbial growth differed over time between the two water types. The pH of acidified bottles (n=4) and autoclaved acidified bottles (n=4) was recorded before and after autoclaving, and on day 7. For sterility testing, samples from day 0 and day 7 were sent to our Veterinary Pathology Program and cultured on blood agar plates. There was little to no change in the pH after autoclaving and over time, and there was no bacterial growth for any sample on day 0 or day 7. To take a step further, day 0 (n=8) and day 7 (n=7) samples were then sent to Charles River Laboratories for full bioburden testing. There was no microbial growth from any sample undergoing bioburden testing as well. These results indicate that there is no need to autoclave acidified water in our facility. However, it is important to note that our facility distributes reverse osmosis water to all animals, and these results might not be consistent for facilities using tap water or other water types instead.

Introduction

At City of Hope, husbandry practices are designed to go above and beyond to ensure animal welfare and maintain consistent research results. As in many animal care programs, some ways we maintain high quality care for our immunodeficient mice include: weekly cage changes, acidified water bottles (autoclaved as well, if by PI request), transporting from cage to cage with forceps, complete PPE, and maintaining overall integrity of the animal rooms by making sure rooms are always clean, objects are disinfected, and appropriate room order is followed (to avoid cross contamination by backtracking into an immunocompromised animal room after being in an immunocompetent room).⁽¹⁾ However, we are always looking for ways to better our practices to ensure animal care remains at a Gold Standard 24/7. With this being said, we wanted to study the types of waters that are used within the facility for these delicate strains. Water bottles are filled with acidified water with a pH of 2.4-3.0 to control for *Pseudomonas* Spp. This water is dispensed from the facility’s reverse osmosis (RO) water system within the cage wash area. The water bottle cases are then sent to animal rooms, where the technicians in each room manually switch out water bottles and uncup every one before sending used water bottles back to cage wash (sometimes over 100 a day).

One previously published study tested water bottles in regards to their quality and sterility over time while being stored in the facility, and it showed promising results⁽²⁾. However, we wanted to take it a step further to ensure the quality and sterility of the water while in the animal’s microenvironment. Given we use two different types of water in our facility for our NSG mice (acidified or acidified followed by autoclaving), we wanted to evaluate any potential differences between the two water types as well.



Purpose and Hypothesis

The purpose of this study was to evaluate the quality and sterility of acidified water and acidified autoclaved water over time when given to NSG mice in our facility. We hypothesized there would be no differences in the pH and/or microbial growth over time between the two water types.

If our hypothesis proves correct, we might be able to eliminate the extra step of autoclaving acidified water, which would lead to better productivity in our cage wash department and a decrease in steam and power usage by our facility.

Methods & Materials

Experiment #1

Test #1: pH check:

- ❖ The pH meter was calibrated following the manufacture instructions before usage. (Checker® pH Test with 0.1 pH Resolution with kit).
- ❖ Two separate orders of opti water bottles containing acidified water were prepared in cage wash. One order was autoclaved. (Water bottles from the Optimice® caging system were filled with RO water from our Edstrom Indigo RO system by our Edstrom bottle filler)⁽³⁾.
- ❖ The pH probe was inserted into each water bottle (acidified n = 4, acidified autoclaved n = 4), and the pH of each bottle was recorded. The pH probe was cleaned using the buffer solution provided in the kit between each usage.
- ❖ Bottles were then placed in a NSG breeding cage. Strain used: NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG): Four cages (Optimice® caging from Animal Care Systems, Inc.) with five resident mice in each.
- ❖ The above procedure was performed before and after autoclaving, once the bottles were cooled and before the bottles were placed in each cage, and at day 7 (when the bottles were replaced).

Test #2: Sterility (In house):

- ❖ All surface areas, objects/supplies, and gloved hands were wiped and sprayed down with **Clidox®**, and all work was done under a Biosafety Cabinet.
- ❖ A 10ml sample of water was collected from each water bottle before being placed in the cage and after 7 days. Samples were transferred to sterile plastic centrifuge tubes (Figure 1) and given to the Center for Comparative Medicine Veterinary Pathology Program for culture.

Culturing:

- ❖ A sterile swab was inserted into the water sample, and a four quadrant streak was performed on a blood agar plate (BAP, Figure 2). (This procedure was conducted for each water sample collected)
- ❖ The culture plates were incubated at 37°C (98.6 °F) for 48hrs.
- ❖ Plates were then checked for bacterial growth at 48 hrs.
- ❖ If isolated colonies were observed, they would have been collected for identification.

Experiment #2

Sterility Testing (Charles River Laboratories):

- ❖ Water samples were collected in provided sterile collection containers (acidified n= 4, acidified autoclaved n = 4, Figure 3) and shipped out to Charles River for full microbial bioburden testing. (Microbial Bioburden water test counts with MALDI-TOF).

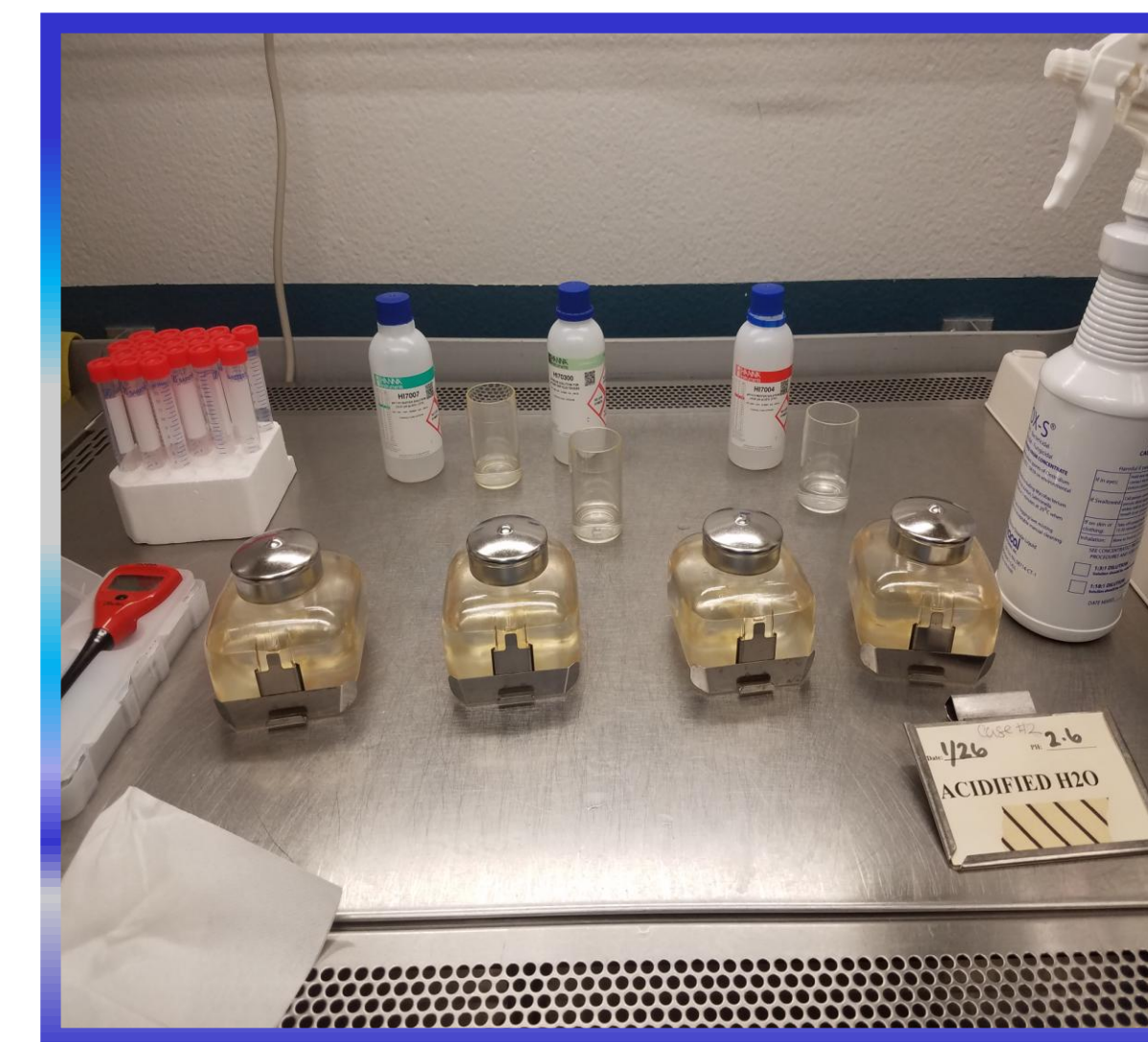


Figure 1. pH testing for waters, in house sterility test tubes (left), Clidox (right).



Figure 2. In house culture testing on BAP with sterile swab.



Figure 3. Bioburden water testing containers from Charles River Laboratories.

Results: pH Levels

When the waters were first made:

- ❖ Acidified water: 2.4
- ❖ Acidified hot “autoclaved” water: 2.1

Day one of experiment (cooled waters):

- ❖ Acidified water: 2.5
- ❖ Acidified “autoclaved” water: 2.4

Day seven of experiment:

- ❖ Acidified water: 2.5
- ❖ Acidified “autoclaved” water: 2.4

Results: Water Sterility

In house testing (pre/post):

- ❖ Negative for aerobic bacteria

Charles River testing (pre/post):

- ❖ Negative for aerobic bacteria, anaerobic bacteria, and fungi



Conclusion

It was indeed concluded that pH levels stayed consistent and microbial growth was absent. These results indicate that both water types are truly sterile (and stay sterile over the life of the bottle in the cage), and there is no need to autoclave acidified water in our facility. Even though our facility strives to go above and beyond when it comes to the wellbeing of all animals (especially immunocompromised strains), it is sometimes necessary to take a step back and stop unnecessarily overanalyzing a process. PI’s request autoclaving on top of acidification of their water due to fear of contamination, however in the end, they are paying for an unnecessary step. Just keep it simple, to the point, and be consistent. In other words...

“Just Let it Flow.”

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