

# Alpha HydroMAID

## Cleaning Effectiveness of the Alpha HydroMAID Cleaning System versus Conventional Mopping

The purpose of these studies was to evaluate the effectiveness of the Alpha HydroMAID (Mobile Automated Integrated Diluter) for removing a bacterial/fungal mixture typical of a kennel floor bio-load and a chemical solution representative of animal urine. The studies were carried out on a typical kennel tiled floor and compared cleaning effectiveness of Alpha HydroMAID with a cotton string mop and a polyester microfiber flat mop. No cleaning surfactants or products containing antimicrobial agents were used in this study. In this way, it was possible to directly measure and compare each cleaning system's inherent ability to remove typical soils.



### Materials and Methods

Prior to testing, the floor was thoroughly sanitized using 70% isopropanol. Both tile surface and grout lines were sampled to ensure effective sanitization. Designated areas of floor were then swabbed with either the microorganism mixture or a chemical creatinine solution, allowed to dry, and sampled on both tile surface and grout lines to determine contamination level prior to cleaning. Each cleaning tool was then applied to a designated area, employing water only as cleaning agent. Samples were then taken following cleaning and either plated to determine microbial counts after cleaning or analyzed for creatinine levels after cleaning. From the data obtained, percentage reduction of contaminants was calculated.



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### Media and Reagents

- a. Trypticase Soy Agar (TSA)
- b. 0.85% Saline
- c. Creatinine, 99+%
- d. Urine Test Strips, micro-albumin and creatinine

### Laboratory Supplies

- a. Standard laboratory glassware and equipment
- b. Sterile 100 x 15 mm petri dishes
- c. Sterile serological pipettes
- d. Sterile cotton swabs
- e. Sterile sponges
- f. Incubator to maintain 35 + 2oC
- g. Vortex mixer
- h. 100 ul pipette

### Additional Equipment

- a. Spray-and-Squeegee touchless cleaning system  
(For the purpose of these studies, an Alpha HydroMAID cleaning system was selected.)
- b. String mops with cotton head
- c. Mops with polyester microfiber head
- d. Deck brushes

### Test Organisms

Escherichia coli ATCC #8739

Enterococcus faecalis ATCC #51299

Enterobacter cloacae ATCC #13047

Staphylococcus aureus ATCC #6538

Salmonella choleraesuis ATCC #14028

Candida albicans ATCC #10231

### Inoculum Preparation

1. Each test organism was transferred from stock culture to TSA and incubated for 18-24 hours at 35 + 2oC. A small loopful of cells was transferred to 10ml TSB and incubated for 18-24 hours at 35 + 2oC.
2. The concentration of each TSB culture was adjusted to approximately 5.0 x 10<sup>8</sup> Colony Forming Units (CFU)/mL.
3. Equal volumes of each organism suspension were mixed (total volume approx. 60ml) in a sterile sample cup to generate an inoculum preparation, which approximates a fecal slurry.
4. The inoculum mixture was serial diluted in sterile saline, and appropriate dilutions were plated in duplicate to determine inoculum concentration.

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### Creatinine Solution Preparation

Creatinine was weighed out and dissolved in tap water at a concentration of 800mg/dL.

### Floor Preparation

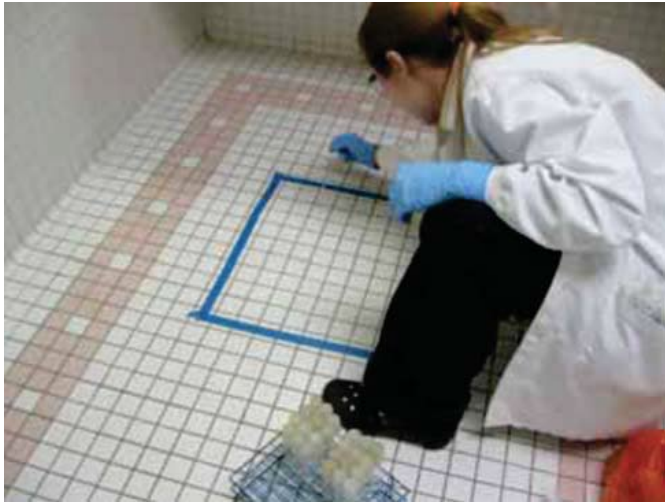
1. Areas of floor each measuring 18" x 18" were marked off.
2. Each area was saturated with 70% IPA and allowed to dry for 10 minutes. Any areas which remained wet after 10 minutes were dried with sterile gauze.
3. Within each area three non-adjacent 2" x 2" tiles and three 4" x 4" intersecting grout lines were selected. To avoid cross-cleaning, tiles and grout lines were selected which were not adjacent or touching.
4. To check for background contamination, sterile cotton swabs moistened in 0.85% saline were used. Each of the three tiles and grout lines were swabbed, and the swabs were immediately placed aseptically into 9ml saline tubes and vortexed.
5. To measure post-sanitization floor contamination level, each swab was diluted and plated in duplicate.

### Inoculation and pre-cleaning analysis of floor

1. A sterile sponge was used to apply the inoculum. A spray bottle was used to apply the creatinine solution over the test area. In each case, care was taken to achieve even coverage and ensure no areas were missed. The sponged or sprayed areas were allowed to dry thoroughly.
2. Three tiles and three grout lines were selected for microbial sampling after the drying period. Microbial swabs were diluted and plated in duplicate to determine post-inoculation count. Two tiles and two grout lines were selected for the simulated urine test. Creatinine levels were determined by the procedure described below.
3. A 25 ul drop of tap water was placed on the spot to be analyzed either on tile or grout using a 100 ul pipette. The drop was mixed with the pipette tip for approximately 20 seconds. The urine test strip pad was then placed into the drop and held for 10 seconds and removed. After a thirty-second reaction time, the color of the pad was compared to the color chart, and the creatinine concentration was determined and recorded.

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Laboratory analysts taking microbial sample with swab



Taking creatinine sample with urine test strip

### Floor Cleaning Techniques

Freshly opened, never used mop heads were used for each trial. The string and flat mop heads were completely submerged in clean tap water and wrung out to a damp state. The floor was mopped in one direction and mopped back in the opposite direction along the same path.

The Alpha HydroMAID cleaning system was used to apply clean water to the floor area using the spray setting. The water was then squeegeed away, one pass in one direction, and a second pass in other.

To sample the tile and grout floor surfaces after cleaning, sterile cotton swabs or urine test strips were used.

### Plate Counting and Calculations

1. Colonies on plates were counted, and CFU per sampled floor area per treatment was calculated.
2. The initial inoculum level for each tile/grout line triplet before cleaning was calculated, and the average per area was calculated.
3. The levels after cleaning for each tile/grout line triplet were calculated, and the average per area was calculated.
4. Finally, the % reduction for each cleaning method was calculated  $(\text{count before cleaning} - \text{count after cleaning}) / (\text{count before cleaning}) \times 100$ . This percentage reduction calculation was used for creatinine reduction as well.

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Bacterial Growth Plate



Urine Creatinine Test Strips

### Results

Cleaning the tiled kennel floor with mops and the Alpha HydroMAID cleaning system showed that it is more difficult to clean a grout surface than a tile surface. For all three pieces of cleaning equipment shown below in Table 1, the percentage reduction in microbial residue on the floor after cleaning was greater on tile versus grout. The Alpha HydroMAID cleaning system demonstrated by far the greater percent microbial reduction on either surface. The mops showed 25-40 % less cleaning capacity on grout versus tile, but the Alpha HydroMAID cleaning system had only about a 2% difference, which cleaned efficiently on both surfaces.

**Table 1.**

Comparison of Tile and Grout Cleaning Efficiency (% reduction in microbial residue on the floor)

Cleaning Equipment	Tile	Grout
Spray-and-Squeegee	99.9	98.1
String Mop	81.0	43.1
Flat Mop	82.2	56.8

The above data clearly shows that grout surfaces are more difficult to clean than tile. To compare the hardest-to-clean grout surfaces in more detail, Figure 1 below shows the microbial counts before and after cleaning on grout for conventional mops and the Alpha HydroMAID cleaning system. The bar graph makes it easy to see that the Alpha HydroMAID cleaning system did a superior cleaning job as compared to the two types of conventional mops. After cleaning, the string mop left 2004 CFU versus the Alpha HydroMAID cleaning system leaving only 55 CFU. Therefore, the string mop left 36 times more bacteria on the grout floor surface than the Alpha HydroMAID cleaning system.



**Figure 1.**  
**Microbial Content on Floor Grout Line Before and After Cleaning**

Cleaning of Microbial Contamination by Spray-and-Squeegee and Conventional Mops

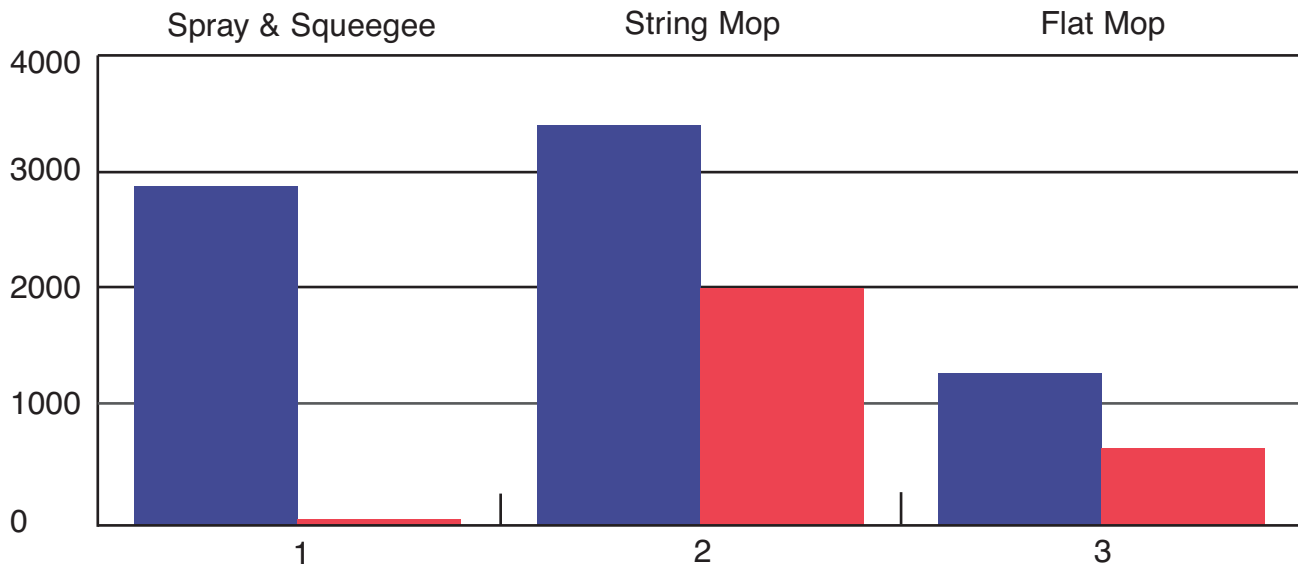


Table 2 below shows that for removing dried biochemical residue such as urine from floor surfaces, damp mopping is significantly inferior to the Spray-and-Squeegee touchless cleaning. The data show that even on a smooth tile surface, the damp mops barely removed half the chemical residue. In contrast, the Alpha HydroMAID procedure removed nearly all of the residue. The data in Table 2 also demonstrate that it is very difficult for string or especially flat mops to clean a chemical residue such as urine off the rough grout surface. The mops removed only about one fifth to one-third of the chemical residue.

**Table 2.**

Comparison of Tile and Grout Cleaning Efficiency (% reduction in simulated urine residue on the floor)

Cleaning Equipment	Tile	Grout
Spray-and-Squeegee	97.4	98.3
String Mop	53.1	38.5
Flat Mop	57.4	21.6

The above data show that grout surfaces are more difficult to clean than tile for simulated urine chemical residue. Figure 2 below displays the creatinine levels representative of dried urine residue before and after cleaning for conventional mops and the Alpha HydroMAID cleaning system. As was the case with the microbial residues, the graph below clearly shows that the Alpha HydroMAID system did a superior cleaning job on grout as compared to the two types of conventional mops. After cleaning, the flat mop left 20.0 mmole/L creatinine residue versus the Alpha HydroMAID system leaving only 0.5. Therefore, the flat mop left 40 times more chemical residue on the grout floor surface than the Alpha HydroMAID cleaning system.

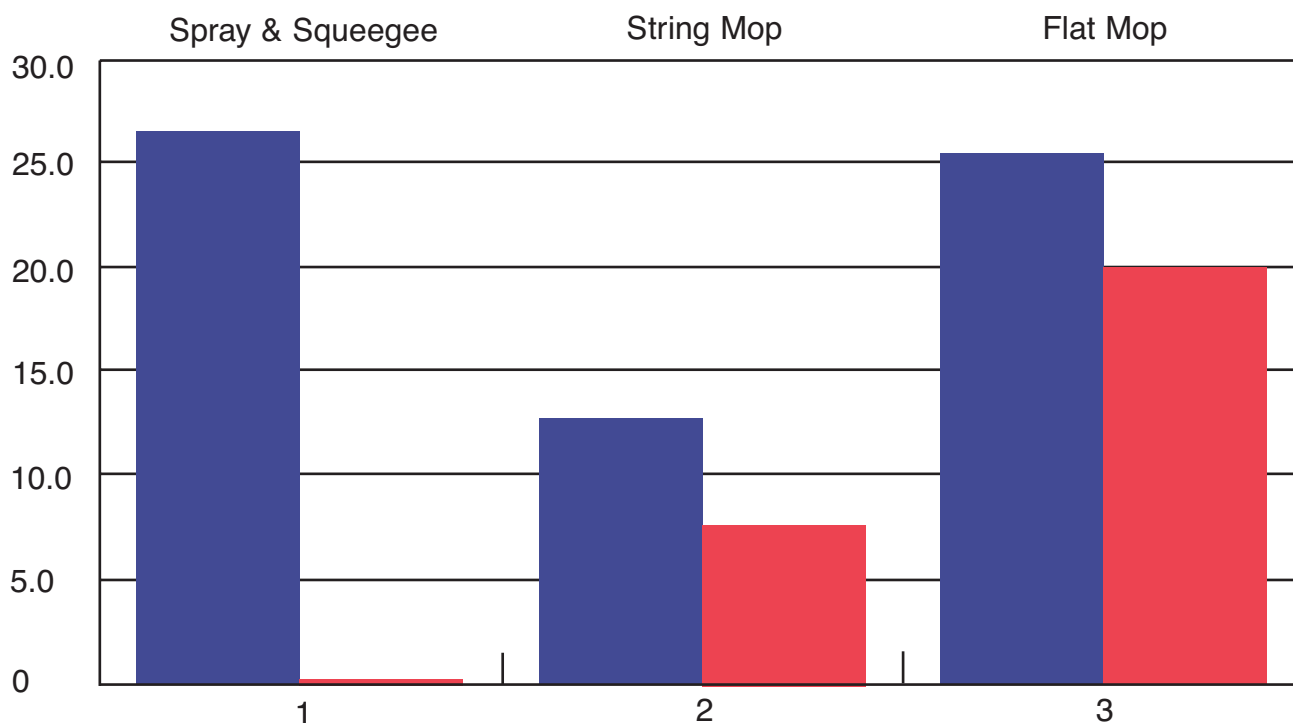
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**Figure 2.**  
**Simulated Urine Content on Floor Grout Line Before and After Cleaning**

Cleaning of Simulated Urine by Spray-and-Squeegee and Conventional Mops



### Discussion and Conclusions

The data generated in this cleaning study clearly shows that the Alpha HydroMAID cleaning equipment does a superior job in removing both microbiological and chemical soils, as compared to conventional mopping. The data shows that for all three pieces of equipment used to clean a tile kennel floor, the grout surfaces are more difficult to clean than the tile surfaces. It appears that the string and flat mops are not capable of efficiently reaching into the valleys of the rough grout surface, but just able to contact the high surfaces. Therefore, much of the soil in the valleys is not removed. In contrast, the Alpha HydroMAID cleaning system demonstrated only a small difference in cleaning efficiency between tile and grout surfaces.

The data also shows that the damp mops were less effective on creatinine chemical residue than on microbial residues. This suggests that damp conventional mopping is not efficient for cleaning and removing dried urine. The difference might be explained by the way in which microorganisms versus chemical solution bind to the floor and especially the rough grout surface. Individual microbial cells make a point contact with the grout by nature of their round shape. In contrast, a chemical solution such as urine or creatinine molds itself as a liquid skin to the exact shape of the floor. When the water evaporates, the chemical becomes a film which coats every contour in the grout, and is much more difficult to remove with transverse movement of the damp mop.

The data indicates that spray and squeegee cleaning is ideally suited for cleaning grout surfaces and urine because excess water from the spray gets into the grout valleys and re-solubilizes the soil so it can be squeegeed away. A damp mop may not contain enough free water to accomplish this.

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For the purposes of this study, virgin mop heads were used, so as to directly and accurately measure only the bio- and chemical loads applied to the pre-cleaned test surface flooring. In the real world, previously-used mops would be the rule, which would add bacterial and chemical loads to the floor during the cleaning process. As a result, spray and squeegee cleaning, which is performed with fresh water each time, would demonstrate an even greater advantage versus mopping than was shown in this study. In addition, as a margin of safety to provide the ultimate cleaning efficiency, a cleaning agent or sanitizer would be used to provide surfactant to better solubilize the soil and anti-microbial chemicals to kill microorganisms.

Overall, the Alpha HydroMAID cleaning system removed 36 times more bacteria and 40 times more chemical residue than the string and flat mops on the hardest-to-clean grout surfaces. Therefore, use of the Alpha HydroMAID cleaning system instead of conventional mops should result in cleaner, fresher-smelling, more sanitary animal care facilities.



### Alpha HydroMAID

(Mobile Automated Integrated Diluter)