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Article

Effects of Temperature on Hand Sanitizer Efficiency

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SUMMARY

In times of pandemic, hand sanitizers are one of the things that slow the spread of contagious disease. As they are readily available, they are stored at different temperatures because they might be used in different environments. It is important to understand what effects storage temperature has on hand sanitizer effectiveness in order to maximize its effectiveness. This research addresses the effect of varying temperatures on hand sanitizer effectiveness. In four separate trials, we applied hand sanitizer before and after touching a cell phone to expose our hands to microbes, then applied our hands to pre-poured sterile LB-agar plates. After 75 hours, we compared the number of surviving bacteria from before and after plates and generated a killing effectiveness value. This study was successful in finding the most effective temperature for 70% ethyl-alcohol-based sanitizers. Our results show that hand sanitizers are the least effective at temperatures above 107.27 °F and the most effective at 96.17 °F. Knowing the temperature range at which sanitizers are the most effective will help us better store the sanitizers to achieve their maximum effectiveness.

INTRODUCTION

Hand sanitizers are known to be one of the effective ways to kill microbes and clean hands. Components that make up sanitizers, such as ethyl alcohol, are known to be affected by environmental factors (1); ethyl alcohol's reactance is dependent on changes in temperature and pressure (2). Although the effectiveness of liquid soap and hand sanitizer against specific viruses, such as Norwalk, and hand sanitizer as an alternative to handwashing have been studied thoroughly previously, little is known regarding the environmental temperature effects on hand sanitizers (3-6).

Since Lupe Hernández invented hand sanitizer gel in 1966, for the past few decades, countries have been developing hand sanitizers (7). Sanitizers can be important in preventing diseases that are more likely to be transmitted via contact hands or by touching contaminated surfaces and objects. For example, rhinovirus and parainfluenza are transmitted through direct contact, and hand sanitation and hand washing can be used to combat these diseases. They are causes of 10–40% of the common cold and parainfluenza sometimes can lead to severe conditions such as pneumonia (8). Further, 22 million absent school days and 20 million absent workdays were due to the common cold in 1996 (9). However, hand sanitizers are normally stored at vastly different temperatures, and it remains unclear how temperature affects hand sanitizer effectiveness; to better inform the public on how to effectively use hand sanitizers to maximize sanitizer effectiveness and prevent the spread of infectious diseases, we need information on how storage temperature affects hand sanitizer effectiveness. We know that change in temperature has effects on the reactance capacitance of liquids (10). Based on this, we hypothesized that storage temperature impacts the nature and effectiveness of the sanitizer in killing bacteria and that the warmer the sanitizer, the more efficient it would be as an increase in temperature leads to an increase in the rate of reaction as the average kinetic energy of the minimum of reactant molecules increases (11).

In this study, we used 70% ethyl alcohol-based hand sanitizers at different temperatures to test this hypothesis. We showed that low temperatures and very high temperatures decrease hand sanitizer effectiveness and we found that 96.17 °F was the most optimal temperature for the best hand sanitizer effectiveness out of the four temperatures assessed.

RESULTS

To test whether warmer hand sanitizer was more efficient at killing microbes, we quantified microbe growth after 75 hours resulting from contaminated hands before and after hand sanitizer application at four different temperatures (45.53 °F, 79.43 °F, 96.17 °F, and 107.27 °F). We compared bacterial growth before applying 107.27 °F hand sanitizer and after applying one drop of 107.27 °F hand sanitizer **(Figure 1)**. We chose four different temperatures to roughly represent the average winter temperature in the Northeast (45 °F), room temperature (79 °F), and vehicle interior temperatures (96 °F and 108 °F) during summer. Before and after applying a drop of hand sanitizer, we absorbed the microbes from a clean hand that has touched a contaminated iPhone 11.

In our comparison of hand sanitizer effectiveness at 45.53 °F, 79.43 °F, 96.17 °F, and 107.27 °F, we found the test group at 96.17 °F had the highest overall effectiveness, based on the number of microbes killed out of the possible total (mean = 93.17%, meaning one drop of sanitizer killed 93.17% of bacteria; SD = 2.97%) **(Figure 2)**. The test group at 107.27 °F had the lowest overall effectiveness (mean = 51.95%; SD = 29.56%). Groups at 45.53 °F

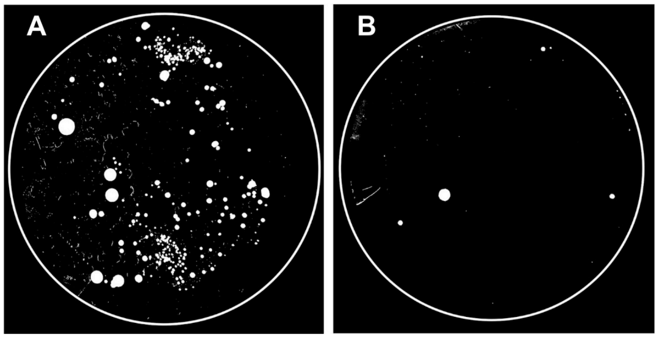

Figure 1: Result after 75 hours in absence of light. Comparison between plate A, before applying 108 °F hand sanitizer, and plate B, after applying one drop of 108 °F hand sanitizer. This shows the visual comparison between bacterial growth before and after applying one drop of hand sanitizer stored at 108 °F. Plates were stored for 75 hours before imaging. Images were taken by iPhone 11's Dual 12MP Ultra-Wide and Wide cameras and ImageJ was used to process the images. Plate A shows 283 colonies with a bacterial area of 142,842 pixel², while plate B shows 9 colonies with a bacterial area of 8,886 pixel².

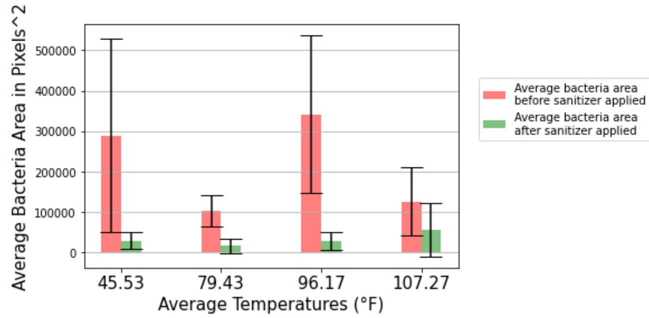

Figure 3: Average bacteria area in pixels² after 75 hours at four different temperatures. The area before hand sanitizer was applied (pink) and after hand sanitizer was applied (green). ImageJ was used to process and calculate the bacteria area in pixels². The average for each condition was taken over three trials. Error bars show standard deviation.

and 79.43 °F had similar effectiveness (mean = 86.44%, SD = 5.08% and mean = 86.50%, SD = 10.43%, respectively) **(Figure 2)**. There is a trending decrease in the effectiveness of hand sanitizer at increasing storage temperature, though it is not statistically significant (p = 0.11, one-way ANOVA, F = 2.73, Bonferroni adjusted α = 0.00833). These results suggest that storing hand sanitizer at temperatures as high as 107.27 °F reduces effectiveness and that sanitizer is most effective at killing bacteria at 96.17 °F.

We compared the effectiveness of each storage temperature against all other possible storage temperatures. We concluded that there is no statistical difference in terms of effectiveness as all t-tests yielded a p-value greater than the Bonferroni adjusted α = 0.00833 (p = 0.994 for 45.53 °F vs 79.43 °F, p = 0.181 for 45.53 °F vs 96.17 °F, p = 0.179 for 45.53 °F vs 107.27 °F, p = 0.433 for 79.43 °F vs 96.17 °F, p = 0.194 for 79.43 °F vs 107.27 °F, and p = 0.121 for 96.17 °F vs 107.27 °F). Next, we compared the average bacteria area after sanitizer was applied to different temperature groups **(Figure 3)**. All t-tests yielded a p-value greater than the Bonferroni adjusted α = 0.0125 (p = 0.201 for 45.53 °F group before vs after sanitizer was applied, p = 0.045 for 79.43 °F group before vs after sanitizer was applied, p = 0.086 for 96.17 °F group before vs after sanitizer was applied, and p = 0.086 for 107.27 °F group before vs after sanitizer was applied) and we concluded that the means of all temperature groups are not significantly different in terms of bacteria area after sanitizer was applied. These statistical analyses suggest that even though there is no statistical difference in terms of effectiveness, a trend is seen suggesting that 79.43 °F is the most effective (p = 0.045) **(Figure 3)**.

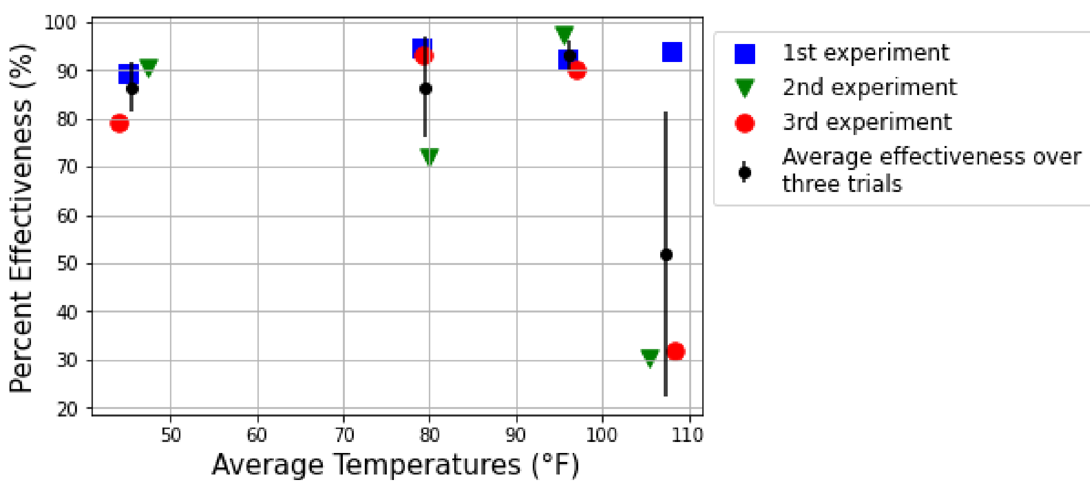

Figure 2: Hand sanitizer effectiveness (%) over three trials plotted against temperature (°F). Skin microbe growth was quantified before and after applying hand sanitizer stored at approximately 45, 79, 96, or 108 °F. With Bonferroni correction, six t-tests were performed comparing the four temperature groups; it was concluded that the means of all temperature groups are not significantly different in terms of effectiveness. Error bars show standard deviation.

Average storage temperature (˚F)	Average effectiveness at killing microbes (%)
107.27	51.95
96.17	93.17
79.44	86.50
45.53	86.44

Table 1: Sanitizer's average effectiveness at four different temperatures. Average effectiveness is calculated over three experiments using Equation 1.

Additionally, we calculated the percent effectiveness for each experiment using the formula from Equation 1 (see Materials and Methods) and then took the average for each group **(Table 1)**. From these calculations, we found that the lowest percent effectiveness is 30.44% and the highest percent effectiveness is 97.21%.

DISCUSSION

Our research addresses the effect of varying temperatures on hand sanitizer effectiveness. By using agar plates, we calculated the effectiveness of varying temperature hand sanitizers. Hand sanitizers reach maximum effectiveness between 79.43 °F and 96.17 °F. It is possible that the sanitizer at 107.27 °F may have burned off a large amount of the microbe-killing substances, including the 70% ethyl alcohol. The 96.17 °F sanitizer may retain high levels of the microbe-killing substance, which allows it to kill off a large number of microbes.

To test our hypothesis that the warmest hand sanitizer would be the most effective at eliminating microbes, we tested four different temperature cases (45.53 °F, 79.44 °F, 96.17 °F, 107.27 °F). We calculated the effectiveness of the sanitizers by using ImageJ to quantify microbe growth on agar plates. Using these results, we calculated the effectiveness of sanitizers by comparing the microbe growth before and after sanitizer addition.

According to our results, we recommend grocery stores and retailers to store hand sanitizers at warmer temperatures, or for customers, to warm up sanitizers before use for high effectiveness. Since the sanitizer works best at a warm temperature, it is best to use it during summer/spring, or preferably, not keep it for more than 20 minutes inside a vehicle to obtain the highest possible effectiveness.

The limitations of this study include a human error when transferring bacteria from hand to plate and the incubator temperature in which we stored the plates. For this experiment, contamination is a big source of human error. To reduce contamination, next time, we will be in a clean room to minimize bacteria transfer and spreading. Also, we will use an incubator with an air sensor that regulates the temperature of the environment inside the incubator, giving all agar plates the same environment to grow in.

Many sanitizers are commonly stored in a vehicle, where the interior air temperature rises dramatically with an increase of 2 °F per minute when the vehicle is turned off (12). Whether this sudden increase in air temperature has an effect on hand sanitizers effectiveness was not directly tested. In 20 minutes, the estimated interior air temperature of the car could be up to 109 °F degrees, at which our data suggest that it may compromise the effectiveness of the hand sanitizer (12).

Based on the results, we theorized hand sanitizers that have been in a car for more than 20 minutes are not at their peak effectiveness. It is advantageous to know an effective way to use hand sanitizers in pandemics, such as the COVID-19 pandemic. In future research, we hope to include more test sets and test effectiveness at more diverse temperatures. We aim our future work on researching how temperature affects hand sanitizers of different concentrations.

MATERIALS AND METHODS

Microbe quantification

We quantified skin microbe growth before and after applying hand sanitizer using ImageJ. For comparison purposes, we tracked the total bacterial area in pixel^2 calculated by the software and used a percent difference formula to calculate the change in the percentage of microbes before and after the addition of hand sanitizer (Equation 1). To get the total bacterial area, we used the "Analyze Particles" in "Analyze" after the "Watershed" in "Process" and the "Threshold" in the "Image" menu.

$$\text{Percent Effectiveness} = \frac{Bacterial\ Area\ Before\ Sanitizer - Bacterial\ Area\ After}{Bacterial\ Area\ Before\ Sanitizer} \cdot 100$$

Equation 1. Percent difference equation used to calculate percent effectiveness.

Software-based measurements

For comparisons, we use a Java-based image processing program, ImageJ, developed at the National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, which estimates the growth of microbes based on the quantification of each individual colony area, in pixels. **Figure 2** represents examples of processed images that we took using iPhone 11's Dual 12MP Ultra Wide and Wide cameras and then analyzed in ImageJ (13).

Hand sanitizer storage & heating

Prior to experiments, we stored all 2 fl oz bottled sanitizers by Lily of the Desert at room temperature of 76 ˚F for the same amount of time. For experiments, we decreased or increased sanitizer temperatures to reach 45.53 °F, 79.43 °F, 96.17 °F, and 107.27 °F. A bottled sanitizer that was previously cooled down or warmed up was never used again for experiments. To reach 96.17 °F and 107.27 °F, 20 oz bottled sanitizers were put in a cup of warm and hot water, respectively, for 10 minutes. To reach 45.53 °F, a separate bottled sanitizer was put in a freezer for 10 minutes. We did not change the temperature of the 79.43 °F sanitizers as we stored them at room temperature of 76 °F. To measure the hand sanitizer temperature with high precision, we used a digital instant reading thermometer with 3.9 inches of stainless-steel probe.

Agar plate production

We bought Pre-poured sterile LB-agar plates (100 x 15 mm Petri Dish) and 10 sterile 6-inch long swabs from EZ BioResearch, 15% aloe content and 70% ethyl alcohol content from Lily of the Desert, and antibacterial bar soap from Safeguard.

Microbe collection and plating

To guarantee the consistency and reliability of the microbe growth across all plates, we washed hands with Safeguard antibacterial bar soap for at least 20 seconds before each trial to avoid introducing bias. For microbe transfer, we rubbed the back of a contaminated iPhone, which we cleaned thoroughly before the first floor contamination, on a clean palm for 10 seconds. Every time before we rubbed the phone, we put the iPhone on a kitchen floor for 10 seconds to “gain” bacteria on its surface. Then, we used a wet 6-inch sterile cotton swab to pick up microbes from the palm for 10 seconds. After that, we rubbed the swab on an EZ BioResearch Bacteria Science Kit (IV) Prepoured sterile Luria Broth Agar Plate for 10 seconds and labeled "Before." By squeezing the bottom bottle, we applied 3 mL, or the recommended dime-sized amount of sanitizer, of Lily of the Desert hand sanitizers, which contain 15% aloe content and 70% ethyl alcohol, to the palm, and the liquid flew through the hole in the flip-top cap (14-15). After rubbing hands until dry, we used a wet swab to collect microbes from the palm and then rubbed on an “after” plate for 10 seconds. We repeated this measurement 3 times at each temperature (45.53 °F, 79.43 °F, 96.17 °F, and 107.27 °F), resulting in a total of 24 measurements. We stored the agar plates for 75 hours at 85 °F. We used ImageJ to report the mean, standard deviation (SD), minimum, and maximum areas of colony-forming units of microbes.

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