The Ability to Affect the Results of a Phenolphthalein Test with Household Chemicals

Javier Moreno & Dr. Adrienne Brundage
Texas A&M University, Department of Entomology
Edited by Jackson Wingert

Abstract: The detection of blood at a crime scene can yield great progress for an investigation. The presence of the bodily fluid can lead to the knowledge of whether the blood is in fact human, the amount of people that were present at the scene, and lead to DNA analysis being done and obtaining the identity of which the blood belongs to. A component in blood, hemoglobin, can break down hydrogen peroxide, making the protein a great focus for presumptive blood tests. For this experiment, a few household chemicals were used to test their ability to tamper with a color indicator Phenolphthalein Test. If the swab turned pink rapidly, this meant the stain came back positive for blood. If no color change occurred, the test could not detect if the substance swabbed was blood. Results obtained showed that household products with certain chemicals and percentage of said chemicals can be used denature the composition of hemoglobin in bloodstains.

Keywords: blood, hemoglobin, presumptive, Phenolphthalein

For crime scene investigation, being able to obtain DNA evidence from any source can be an essential to solving a case. Whether that evidence is found in saliva, hair, skin, blood, etc., having DNA as a place to start can begin investigations on a good note. While the evidence can yield amazing results, it is possible that factors can be included to cause the ability to move forward in the investigation to halt. Perpetrators may attempt to tamper with the evidence with whatever is easiest to use, and household products lying around are some of the easiest to obtain.

When searching for which products may work best, looking at the chemicals and ingredients used in said products is the best way. Studies from other experiments show that sodium hypochlorite (NaClO) can mess with DNA concentrations in a solution (Edler 2020). Others have tested to see if household cleaners could tamper with blood enough to no display chemiluminescence from a bloodstain (James 2020). Results from the chemiluminescence study showed that none of the chemicals used could affectively tamper with the blood enough to not show with luminol. Finding out that sodium hypochlorite has some ability to tamper with DNA concentrations and curiosity if household products may be able to tamper with evidence brought the interest to test if products containing NaClO may bring a negative result from a presumptive Phenolphthalein test.

Materials and Methods
Blood Sample and Phenolphthalein Test
240 mL bottle of synthetic blood (MEDTECH Forensics, Tallahassee, FL) was acquired from Dr. Brundage. Also acquired was a Phenolphthalein test, consisting of distilled water, an alcohol reagent, hydrogen peroxide, and Phenolphthalein reagent (30 mL bottles).

Bleaching Agents
Four household products were used in the experiment. 22 fl. oz. Oxy Stain Destroyer and Advance Professional Oxy Stain Destroyer Pretreat, Pet Pretreat or Blotter
(BISSELL Homecare, Inc., Grand Rapids, MI): Contains ≤ 3% Hydrogen Peroxide (H₂O₂), ≤ 1% Alkyl polyglucoside, and ≤ 1% Alkoxylated alcohol. 32 fl. oz. Up and Up All-Purpose Cleaner with Bleach (KIK International LLC, Ontario, Canada): Contains 2% Sodium Hypochlorite (NaOCl), 98% other ingredients including water (H₂O), Sodium Hydroxide (NaOH), and Lauryl dimethylamine Oxide (C₁₄H₃₁NO). 32 fl. oz. Liquid-Plumr Clog Destroyer Plus+ Pipeguard (The Clorox Company, Oakland, CA): Contains 0.5-2% NaOH, 5-10% NaOCl, and other ingredients such as H₂O, Sodium Chloride (NaCl), and Cetyl betaine (C₂₁H₄₃NO₂). 43 fl. oz. Clorox Disinfecting Bleach (The Clorox Company): 7.5% NaOCl, and 92.5 % other ingredients including NaCl, Sodium Carbonate (Na₂CO₃), and NaOH.

Experiment

Negative and Positive Control. To dispense the chemicals and create the blood stains, 12 oz. food storage bottle (OXO, Petersburg, VA) was used. Bloodstains and chemicals were poured onto a cut up 100% cotton yellow shirt. Five pieces of the shirt were used, one for each of the bleaching agents and one for the control. Cotton swabs (Unilever, London, England) were used to take a sample of the blood from the shirt and bring the reagents on. 100 mL of the synthetic blood was put into the storage bottle and approximately 20 mL was poured onto each shirt piece. Blood sample was allowed to get a bit dry for about 20 minutes. During the allotted time, a negative control was taken from each shirt piece. Distilled water was added to a cotton swab then used to “collect” from an unaffected area on the shirt. To conduct the Phenolphthalein test, one drop of alcohol is added to the sample area on the swab. Following that should be one drop of Phenolphthalein reagent, then one drop of hydrogen peroxide. The positive control consisted of swabbing the stain with no chemical added to it. The same procedure was done to test the positive control.

Bloodstain Tests. Once the bloodstains had time to dry up, 15 mL of each product was placed in the storage bottle and poured onto the stain. 15 minutes was given for the product to fully spread out on the stain and dry up. After the time passed, the procedure done for the negative and positive controls was done the same for the stain tests.

Results

Testing of the bloodstains with the presumptive test showed that household products may be used to degrade hemoglobin in a blood sample. The negative and positive controls were successful in the expected outcomes. Negative controls which were taken from uncontaminated sections of the shirt yielded no color change. The positive control taken from the bloodstain with no product added to it quickly changed to pink with the presumptive test (Picture 1). The controls help in knowing that the test is running successfully and resulting in what we know should happen.

All tests with products added yielded the same positive result except one. Use of the Clorox Disinfecting Bleach caused a negative result for the presumptive test (Picture. 2). The other three products tested still brought a pink color change (Pictures 3-5). Only one of the chemicals causing a negative result for blood meant that 25% of the products used in the experiment can degrade a blood sample enough to tamper with the test while the remaining 75% still came back positive.
Table 1. Results for Phenolphthalein Tests indicating if a color change occurred.

<table>
<thead>
<tr>
<th>Household Product</th>
<th>Negative Control Result</th>
<th>Positive Control Result</th>
<th>Bloodstain Swab Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxy Stain Destroyer</td>
<td>No color change</td>
<td>N/A</td>
<td>Rapid color change/Pink</td>
</tr>
<tr>
<td>All-Purpose Cleaner with Bleach</td>
<td>No color change</td>
<td>N/A</td>
<td>Color change/Pink</td>
</tr>
<tr>
<td>Liquid-Plumr Gel</td>
<td>No color change</td>
<td>N/A</td>
<td>Rapid color change/Pink</td>
</tr>
<tr>
<td>Clorox Disinfecting Bleach</td>
<td>No color change</td>
<td>N/A</td>
<td>No color change*</td>
</tr>
<tr>
<td>Control</td>
<td>No color change</td>
<td>Rapid color change/Pink</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*No color change indicates that unlike the rest of the products tested, the Clorox Bleach did not result positive for blood.

Fig. 1 Positive control result
Fig. 2 Negative result from Clorox Bleach sample

Fig. 3 Positive result from All-Purpose Cleaner with Bleach sample
Fig. 4 Positive result from Oxy Destroyer sample

Fig. 5 Positive result from Liquid-Plumr Gel
Discussion
The use of bleaching agents to contaminate DNA evidence from a crime scene brings rise to a big problem in obtaining good profiles from bodily fluids, such as blood, for investigations (Passi 2012). The assumption that products containing certain amounts of certain chemicals, such as sodium hypochlorite, may be able to tamper with a presumptive blood test such as Phenolphthalein test.

Results from the experiment show that there could be a correlation with the degradation abilities of the chemical and the presence of sodium hypochlorite. Using the Clorox Bleach that had 7.5% of NaClO resulted in a negative result for blood and the All-Purpose Cleaner with Bleach with 2% NaClO took more time for a color change to occur than the other two products. It appears household products with decently high percentages oxidizing chemicals can contaminate blood samples and in turn the ability to gain a positive profile from that evidence.

References