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TECHNICAL NOTE

CRIMINALISTICS

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Chemistry in Crime Investigation: Sodium Percarbonate Effects on Bloodstains Detection

ABSTRACT: Chemistry plays a leading role in crime investigation. In the study of bloodstains, chemical reactions provide the means for the detection. All these procedures have been thoroughly studied. However, recently, a new source of error has been found: washing stains with "active oxygen" detergents abrogates presumptive and human hemoglobin tests for bloodstains (although visible). The aim of this investigation was to evaluate the ability of pure sodium percarbonate—main component of detergents—to abrogate presumptive and human hemoglobin tests. Then, a solution to this problem could be found. The results demonstrate that pure sodium percarbonate—itself—is able to abrogate all tests, as well as the different degrees to which each of them is affected by the product. Consequently, faced with a stain of bloody appearance, even the preliminary tests are negative; it is advisable to analyze the DNA. Otherwise, the opportunity of obtaining valuable information is lost.

KEYWORDS: forensic science, criminalistics, bloodstains investigation, presumptive test, human hemoglobin test, forensic chemistry

In October 2002, the Royal Society of Chemistry awarded an honorable and posthumous medal to the famous detective Sherlock Holmes. This was the first time that a fictional character had received an award of this type, the reason being his intelligent use of chemistry in his investigations.

There is nothing new, therefore, in claiming that chemistry plays a fundamental role in criminal investigations. On one hand, chemistry is involved in analyzing clues from the crime scene so as to reveal unusual characteristics and, on the other hand, it is responsible for developing new procedures for searching out and analyzing clues. Coming up with ideas for new methods or improving those already existing is undoubtedly an important part of the work of the forensic chemist.

It is these specialists who, as experts in the chemical properties of materials and the reactions that take place between different substances, are best qualified to design the most effective procedures for attending to the properties of the elements to be identified. It was, after all, a simple oxidation–reduction reaction that provided the setting-off point for developing the blood detection methods—for visible and latent stains—still in use today (1,2).

These methods have been thoroughly studied. The possible causes of false positives and negatives (3–6), as well as the ways of trying to prevent them (7,8) are well known. Recently, however, a new and worrying source of error has come to light caused by the use of detergents containing the so-called active oxygen (or sodium percarbonate).

In a study published in 2009 (9), it was concluded that bloodstains washed with these detergents and still visible following the wash provide a negative result in the most commonly used presumptive tests (reduced phenolphthalein, Luminol, and Bluestar® Forensic), as well as for the lateral flow immunochromatographic test for the detection of human hemoglobin. This fact has had a considerable impact on the forensic community as, until now, it was not thought possible that a visible stain (and, therefore, with a sufficient concentration of blood) would not give a positive result for the presumptive test. However, in 2010, an article showed that the washing of bloodstains with active oxygen does not impede DNA analysis (10). In this work, multiplex PCR with the AmpflSTR Profiler PCR amplification kit (Applied Biosystems, SA, Madrid, Spain) was successfully performed. Consequently, the inefficacy of the presumptive test to identify or locate bloodstains means no DNA can be extracted, and therefore, important evidence may be lost. It is, for that reason, essential to find a solution to this problem and the first step to finding that solution is, of course, to discover and determine how the interference of the contaminant comes about.

The research described in this article is aimed at evaluating the ability of soluble sodium percarbonate to abrogate presumptive tests (reduced phenolphthalein, Luminol, and Bluestar® Forensic), and the human hemoglobin test. The work method is as follows.

Materials and Methods

Materials

Sodium percarbonate (PANREAC QUÍMICA S.A.U., Barcelona, Spain) was used to prepare sodium percarbonate solutions. For presumptive test, Luminol (3-aminophthalhydrazide; MERCK-VALENCIA, Valencia, Spain), sodium perborate (PANREAC QUÍMICA S.A.U.), sodium carbonate (PANREAC QUÍMICA S.A.U.), distilled water, Phenolphthalein Dischaps™ (Sirchie Cat. No. DCB100; Madrid, Spain), and Bluestar® Forensic (Seidden Identificación, Madrid, Spain) were used. Human hemoglobin test was carried out using Hexagon OBTI® test (Seidden Identificación).

Sample Preparation

Bloodstains were prepared using newly extracted blood with no preservatives. Stains were made on pieces of white cotton cloth of

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approximately 5 × 5 cm. Each stain was made with three drops of blood. Having made the stains, they were left to dry for over 24 h at room temperature without any protection whatsoever.

To evaluate the effect of sodium percarbonate on the forensic tests, the stains were washed with a solution of this chemical compound. In this way, the possible contribution of the other components of the detergent was eliminated.

The concentration of the sodium percarbonate solution was calculated from the information stated on the detergent label, so determining the interference (Neutrex™; Henkel [http://www.henkel.es/cps/rde/xchg/henkel_ess/hs.xsl/index.htm]). The amount of product recommended for hand washing is 80 g of detergent for 4 L of water. This involved a concentration of 30% of sodium percarbonate.

To wash the stains, an initial solution was prepared by diluting 0.3 g of sodium percarbonate in 50 mL of distilled water (solution concentration, 6 g/L). By doing so, a reagent concentration equivalent to that of the original product was obtained. Depending on the results obtained in the tests carried out with this solution, other less concentrated solutions would be prepared.

Stain Washing

Bloodstains are divided into four groups: A, B, C, and D. Prior to this, several samples are separated so as to be used as positive controls, as well as fragments of white cotton cloth without stains for carrying out negative controls.

Then, "A" stains were washed with the sodium percarbonate solution at 40°C (following the washing instructions indicated on the detergent), for 2 h. "B" stains were washed with the sodium percarbonate solution in water at room temperature (19°C) for 2 h, "C" stains were washed with hot water (at 40°C) for 2 h and, finally, "D" stains were washed with water at room temperature (19°C) for 2 h.

Pieces of white cotton cloth without stains were washed in the same conditions described for A, B, C, and D samples, as negative control. All of them were left to dry for 24 h.

Each stain was then divided into four portions, three to be used in applying the presumptive tests (reduced phenolphthalein, Luminol, and Bluestar® Forensic) and the fourth to be used for the human hemoglobin test.

Presumptive Test

Reduced phenolphthalein test was carried out using a preprepared reagent contained in an ampoule, following the manufacturer's instructions (11).

First, prints were obtained by moistening filter paper with distilled water and pressing it onto the stain. Then, the test was performed on the filter paper. If there was no positive reaction, the process was repeated, applying the reagent directly onto the bloodstain.

Luminol was prepared in accordance with the Grodsky formula (12). This was performed in a darkroom. The reagent was then sprayed onto the stain. The test is considered positive when luminescence is observed a few seconds after having applied the reagent.

Bluestar® Forensic reagent was prepared in accordance with the manufacturer's instructions (13). Like Luminol, Bluestar® Forensic requires work in a darkroom, where the reagent is applied using a spray gun on the stain.

All tests (reduced phenolphthalein, Luminol, and Bluestar® Forensic) were undertaken on stain groups A, B, C, and D, and also on the unwashed bloodstains (positive control) and on pieces of white cotton cloth without stains (washed in the same conditions described for A, B, C, and D samples, as negative control).

Tests were considered positive when the coloration (in the case of the reduced phenolphthalein) or luminescence (Luminol and Bluestar® Forensic) was observed a few seconds after applying the reagent. A slower reaction may be a result of the oxidation of the reagent by agents other than the peroxidases-like activity of the sample.

Human Hemoglobin Test

The Hexagon OBTI® test kit—widely used in forensic laboratories—was used. The test was performed following the manufacturer's instructions (14,15), and the same procedure was applied on all the control samples.

The test is regarded as positive when—as stated in the manufacturer's instructions—the blue test line (indicating the positive result) is formed in the first 5 min following the beginning of the test. Negative results must be confirmed at 10 min.

If, having completed these tests, negative results are obtained on any of the tests undertaken, the procedure is repeated using less concentrated solutions of sodium percarbonate (1/2, 1/4, 1/8, 1/16 of the initial one) for the wash.

Results

The results are shown in Tables 1 and 2 (for negative controls), where a positive test is represented as "++" when the reaction is intense and with "+" when slight; a negative test as "−."

Of particular interest from the data obtained are the following findings. Luminol and Bluestar® Forensic tests provide positive results from sodium percarbonate concentration lower than $1 \cdot 10^{-2}$ M. However, the tests with reduced phenolphthalein as well as the human hemoglobin test are only positive when the concentration is reduced to 1/16 of the initial amount.

It is observed that the wash temperature with the sodium percarbonate solution affects the different tests in different ways. In cold water and for all the concentrations studied, Luminol, Bluestar® Forensic, and reduced phenolphthalein prove positive, although the reaction for the latter is weak. In contrast, the human hemoglobin test proves negative at the highest concentration of sodium percarbonate and only when it is reduced to half the amount does the expected positive result appear.

Samples C and D—washed with hot water (40°C) and cold water (19°C)—without sodium percarbonate, provide positive results for all tests performed.

Negative controls made on pieces of white cotton cloth without stains were washed in the same conditions described for A, B, C, and D samples and provide negative results for all tests. Consequently, sodium percarbonate in hot or cold temperature water does not produce false positives.

Discussion

The results show that washing with a solution of sodium percarbonate ($2 \cdot 10^{-2}$ M; equivalent to the concentration that a wash with detergent has) and at 40°C abrogates all the presumptive tests as well as the human hemoglobin test.

On reducing the concentration of the reagent, it is observed that Luminol and Bluestar® Forensic tests begin to provide positive results before that of the reduced phenolphthalein and human hemoglobin tests. The latter two, therefore, are more sensitive to the effect of sodium percarbonate.

TABLE 1—*Results of presumptive and human hemoglobin test on bloodstains washed with solutions of sodium percarbonate to different concentrations.*

	Samples											
	A					B					C	D
Concentration of Sodium Percarbonate	1	1/2	1/4	1/8	1/16	1	1/2	1/4	1/8	1/16	0	0
Test												
Reduced phenolphthalein	−	−	−	−	+	+	+	++	++	++	++	++
Luminol	−	−	++	++	++	++	++	++	++	++	++	++
Bluestar®	−	−	++	++	++	++	++	++	++	++	++	++
Human hemoglobin	−	−	−	−	+	−	+	++	++	++	++	++

1 indicates a solution concentration of 6 g/L (initial solution); A, washed with sodium percarbonate (40°C); B, washed with sodium percarbonate (22°C); C, water (40°C); D, water (19°C); ++, positive (intense reaction); +, positive (slight reaction); −, negative.

TABLE 2—*Results of presumptive and human hemoglobin test on pieces of white cotton cloth without stains, as negative control.*

	Negative Control Samples											
	A					B					C	D
Concentration of Sodium Percarbonate	1	1/2	1/4	1/8	1/16	1	1/2	1/4	1/8	1/16	0	0
Test												
Reduced phenolphthalein	−	−	−	−	−	−	−	−	−	−	−	−
Luminol	−	−	−	−	−	−	−	−	−	−	−	−
Bluestar®	−	−	−	−	−	−	−	−	−	−	−	−
Human hemoglobin	−	−	−	−	−	−	−	−	−	−	−	−

1 indicates a solution concentration of 6 g/L (initial solution); A, washed with sodium percarbonate (40°C); B, washed with sodium percarbonate (22°C); C, water (40°C); D, water (19°C); ++, positive (intense reaction); +, positive (slight reaction); −, negative.

The results obtained from the samples washed only with hot or cold water (C and D samples) indicate that water temperature alone does not cause any interference on the tests studied. However, it is still a factor to be considered when used in combination with sodium percarbonate, given that together their effectiveness as a contaminant is increased.

From the data provided by this study, it can be seen that sodium percarbonate, an ingredient of washing products "with active oxygen," causes damage to bloodstains so hindering their identification through the procedures that are currently preferred for use in forensic analysis. Consequently, bloodstains, although visible, may be discarded as not relevant in the investigation into a criminal event, hence losing the possibility of obtaining a genetic profile.

In conclusion, a thorough study of the chemical mechanism by which the interference of sodium percarbonate takes place is required as a first step to finding a solution to this problem. Meanwhile, faced with a stain of bloody appearance, even though the presumptive and human hemoglobin tests are negative, it is advisable to try to extract and analyze the DNA. Otherwise, the opportunity of obtaining valuable information for solving a criminal case may be lost.

References

1. Adler O, Adler R. Über das Verhalten gewisser organischer Verbindungen gegenüber Blut mit besonderer Berücksichtigung das Nachweises von Blut. Hoppe-Seyler's Zeitschrift fur Physiologiste Chemie 1904;41:59–67 [article in German].
2. Proescher F, Moody MA. Detection of blood by means of chemiluminescence. J Lab Clin Med 1939;24:1183–9.
3. Cox M. A study of the sensitive and specificity of four presumptive tests for blood. J Forensic Sci 1991;36:1503–11.
4. Barni F, Lewis SW, Berti A, Miskelly GM, Lago G. Forensic application of the luminol reaction as a presumptive test for latent blood detection. Talanta 2007;72(3):896–913.
5. Castelló A, Verdú F. Critical revision of presumptive tests for bloodstains. Forensic Sci Commun 1999;1, http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/july1999/ponce.htm/ (accessed November 16, 2011).
6. Johnston E, Ames CE, Dagnall KE, Foster J, Daniel BE. Comparison of presumptive blood test kits including hexagon OBTI. J Forensic Sci 2008;53(3):687–9.
7. King R, Miskelly GM. The inhibition by amines and amino acids of bleach-induced luminol chemiluminescence during forensic screening for blood. Talanta 2005;67(2):345–53.
8. Castelló A, Francés F, Verdú F. Bleach interference in forensic luminol tests on porous surfaces: more about the drying time effect. Talanta 2009;77(4):1555–7.
9. Castelló A, Francès F, Corella D, Verdú F. Active oxygen doctors the evidence. Naturwissenschaften 2009;96(2):303–7.
10. Castelló A, Francès F, Verdú F. DNA evidence uncompromised by active oxygen. Scientific World J 2010;10:387–92.
11. http://www.sirchie.com/support/D-technical-download.html (accessed January 10, 2011).
12. Grodsky M, Wright K, Kirk PL. Simplified preliminary blood testing. An improved technique and a comparative study of methods. J Crim Law Criminol Police Sci 1951;42:95–104.
13. http://www.bluestar-forensic.com/ (accessed January 10, 2011).
14. http://www.bluestar-forensic.com/pdf/en/hexagon_obti_verif_report.pdf (accessed January 10, 2011).
15. http://www.bluestar-forensic.com/gb/documentation_hexagon.php (accessed January 10, 2011).

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