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Technical Note

A Potential Source of Difficulty in the Initial Testing for Blood

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Introduction

The threats of chemical and biological attack have lead to remediation efforts using various chemical oxidants (Raber and McGuire 2002). Among the new reagents being tested is Oxone, potassium peroxymonosulfate, a product of Dupont, Wilmington, Delaware. This compound has been tested on contaminated substrates with encouraging results, but its effects on subsequent tests of forensic interest still need to be determined (Tumosa et al. 2002).

The initial examination of bloodstains usually includes at least one test based on the catalytic nature of hemoglobin (Gaensslen 1983). In particular, a test based on the oxidation of benzidine or more recently tetramethylbenzidine in the presence of peroxide is often used. The similar phenolphthalin (reduced phenolphthalein) test is also common. Because these tests are based on the oxidation of the reagent, interferences that yield false-positive reactions are often oxidizers. Iron and copper oxides as well as permanganates, sodium hypochlorite, nitric acid, and ferrocyanides have been reported to interfere with the test (Garner et al. 1976; Hunt et al. 1960). Other interferences have been addressed as well (Cox 1991; Gaensslen 1983).

The active ingredient of Oxone is potassium peroxymonosulfate. The salt is present as a component of a triple salt, $2KHSO_5$ $KHSO_4$ K_2SO_4, potassium hydrogen peroxymonosulfate [CAS-RN 70693-62-8]. Oxone is a mild oxidizing agent and has been evaluated for the mitigation of the effects of biological and chemical warfare agents, as well as some viral agents (Raber and McGuire 2002). In addition, other oxidizing compounds, such as sodium perborate and sodium percarbonate, are present in commonly available cleaning and disinfecting products. These compounds were tested for reactions with the 3,3',5,5'-tetramethylbenzidine (TMB) and phenolphthalin reagents as well.

Materials and Methods

The tests were performed in a two-step process. The test reagent was added first, the color of the solution was noted, and then the oxidizing reagent was added. The TMB reagent was prepared as a two-percent solution in glacial acetic acid, and a three-percent hydrogen peroxide solution was used in the second step of the test (Lee 1982). The phenolphthalin reagent (Kastle-Meyer reagent) was prepared by reducing phenolphthalein with zinc metal in basic solution and an approximately 20-percent sodium perborate solution was used in the second step of the test (Culliford 1971). The tests were performed by adding two drops of TMB or phenolphthalin reagents to similar volumes of test material (oxidizing agent), and the color of each was noted after one minute. The three-percent hydrogen peroxide or 20-percent (weight/volume) sodium perborate was then added and the color and time-to-color development was noted.

Solutions of sodium percarbonate, sodium perborate, and Oxone were made to one percent (weight/volume) in deionized water.

The influence of blood in contact with the oxidizing agents was also investigated. The same procedure as above was followed but with human or pig blood on solid substrates, such as filter paper or cotton swabs. Dried samples of human and pig blood of known age, from one week to three years old, on filter paper, cotton swabs, or as crusts were used to test the reagents and to act as controls. Negative controls (both test reagents only) and positive controls (both test reagents with blood) reacted as expected.

All chemicals were of analytical grade, and test reagents were prepared fresh before use.

Results

3,3',5,5'-tetramethylbenzidine reagent

The one-percent Oxone solution reacted with the tetramethylbenzidine reagent, first to give a purple precipitate, then a green color, and finally a yellow solution. This color change also occurred when the one-percent Oxone was dried on cotton swabs and the test reagent added to the swabs. In neither case did the developed color mimic that formed when the TMB reagent and hydrogen peroxide reacted with human or pig blood. This was true whether or not the blood was tested on a cotton or filter paper substrate, as crusts, or dissolved in saline solution. Because the TMB test reagent alone gave color changes with the Oxone, further tests with blood were not performed.

The one-percent sodium percarbonate solution did not react with the tetramethylbenzidine reagent, as might be expected. In the presence of blood with no other peroxide source added, the characteristic color of the test developed quickly. The percarbonate acted as a source of peroxide and functioned as a substitute for the commonly used hydrogen peroxide in the tests. Solutions of sodium percarbonate in contact with visible blood stains evolved bubbles of oxygen gas, as do three-percent solutions of hydrogen peroxide. The sodium percarbonate solution when air dried on swabs failed to react to give the characteristic color (or any color) in the presence of blood and the test reagent alone. The addition of peroxide gave the characteristic color.

Phenolphthalin reagent (Kastle-Meyer reagent)

The one-percent Oxone solution reacted very slowly with the phenolphthalin reagent alone to give a faint pink color after about one minute. This color did not intensify even after five minutes. The color was neither as intense nor as immediate as it would be with an authentic blood sample. In the presence of blood, the color was intense and immediate without the further addition of perborate or hydrogen peroxide.

Sodium percarbonate solutions did not react with the phenolphthalin reagent alone but gave the characteristic red color if blood were present whether in solution or on cotton substrates.

Discussion and Conclusions

Oxone in aqueous concentrations typically used for the decontamination of surfaces will react with the tetramethylbenzidine reagent as used in common forensic practice. The initial colored compound and the color of the ultimate reaction solution are unlike those encountered with authentic blood samples. The phenolphthalin reagent reacted slowly but not in the manner of an actual blood stain, and so the test results should not be mistaken for blood.

Sodium percarbonate in the presence of blood could give a positive-color test for blood without the addition of the usual developer peroxide.

References

Cox, M. A study of the sensitivity and specificity of four presumptive tests for blood, *Journal of Forensic Sciences* (1991) 36:1503-1511.

Culliford, B. J. *Examination and Typing of Bloodstains in the Crime Laboratory*. U.S. Department of Justice, Washington, DC, 1971, pp. 41-51.

Gaensslen, R. E. *Sourcebook in Forensic Serology, Immunology, and Biochemistry*, U.S. Department of Justice, Washington, DC, 1983, pp. 101-116.

Garner, D. D., Cano, K. M., Peimer, R. S., and Yeshion, T. E. Evaluation of tetramethylbenzidine as a presumptive test for blood, *Journal of Forensic Sciences* (1976) 21:816-821.

Hunt, A. C., Corby, C., Dodd, B. E., and Camps, F. E. Identification of human blood stains: A critical survey, *Journal of Forensic Medicine* (1960) 7:112-130.

Lee, H. C. Identification and grouping of bloodstains. In: *Forensic Science Handbook*, R. Saferstein, ed. Prentice Hall, Englewood Cliffs, New Jersey, 1982, pp. 272-276.

Raber, E. and McGuire, R. Oxidative decontamination of chemical and biological warfare agents using L-Gel, *Journal of Hazardous Materials* (2002) B93:339-352.

Tumosa, C. S., Erhardt, D., and Solazzo, C. Effect on ballpoint pen and marker inks of chemical and electron beam remediation techniques for biological warfare agents, *Mid-Atlantic Association of Forensic Scientists Newsletter* (2002) 30(3):5-8.