# The Effects of Blood Enhancement Chemicals on Subsequent DNA Analysis

*(Reprinted from volume 24 (3) of Identification Canada, 2001. Identification Canada published an adapted version from the Journal of Forensic Sciences 45 (2), copyright American Society for Testing and Materials, West Conshohocken, PA.)*

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**Abstract**

Forensic Identification Specialists are often forced to make a decision at a crime scene as to what evidence should be collected, and with which technique. When confronted with indications of weak bloodstains, the choice is between using a blood enhancement reagent to try to bring up fingerprint friction ridge detail, and swabbing the stain for later DNA analysis. The present study indicates that DNA profiles can still be obtained after the use of any of the seven blood enhancement techniques used here, without altering the DNA results. The intensity of the fluorescent signals was very similar and the allele size measurements remained constant and identical to those from untreated blood. Only two issues were noted: 1) a reduction in DNA recovery after blood enhancement in specific cases and, 2) a suggestion of slight degradation of DNA after prolonged exposure to blood reagents which may require special consideration.

**Introduction**

Forensic Identification Specialists are always confronted at the crime scene with having to make a decision regarding which fingerprint development method should be used in a particular situation. Certain techniques can be used after another has been unsuccessful, while some techniques must be used before all others [1] . The problem is more complicated when there is the possibility of recovering body fluids for later DNA analysis. While forensic serological analyses required a large sample to be collected to yield a successful result, DNA profiling has become more and more sensitive, requiring much smaller sample sizes. Now, when a weak mark in blood is detected at the crime scene, a decision has to be made between using a blood enhancement technique to develop friction ridge detail, and swabbing the area for later DNA analysis. The present study aims to provide some direction with respect to these cases [2,3, and references therein].

When serological testing was still in vogue, it was found that fingerprint techniques did have a deleterious effect on ABO or polymorphic enzyme testing [4-6] .With the advent of restriction fragment length polymorphism (RFLP) DNA profiling (considered to be the first common conventional DNA analysis procedure), further studies showed adverse effects from some presumptive tests for blood (silver nitrate, benzidine, leucomalachite green, o-tolidine) [5,7], while certain fingerprint development techniques did not compromise the subsequent DNA analyses [5,8]. Hochmeister et al. [7] reported successful RFLP typing of blood after treatment with luminol, benzidine, and phenolphthalein. Stein and colleagues [9] exposed bloodstains to cyanoacrylate, ninhydrin, and gentian violet for 14 days, and were still able to obtain DNA results.

The use of polymerase chain reaction (PCR) techniques for short tandem repeat (STR) DNA analyses has made DNA profiling much more sensitive. Smaller size samples or degraded samples can still produce full DNA profiles [10-12]. Hochmeister et al. [13] were able to successfully obtain PCR-based results from bloodstains after treatment with cyanoacrylate, dyes, and examination under intense forensic light sources. Stein et al. [9] examined the effects of fingerprint powder, cyanoacrylate, gentian violet, and ninhydrin, and found that DNA profiles could be obtained even 56 days after fingerprint treatment. Andersen and Bramble [14] looked at the results after exposure to various forensic light sources, and found that exposure to shortwave UV light had a damaging effect on subsequent PCR DNA analysis.

The present study looked at seven blood enhancement techniques applied to various surfaces to determine their effect on PCR DNA analysis. Blood drops and bloody fingerprints of various concentrations were used. In some cases, the blood samples were aged before treatment with the enhancement technique, and in others, the samples were left to stand after chemical treatment, before DNA analysis.

**Methods and Materials**

Blood samples from two volunteers were collected in vacutainers containing the anticoagulant EDTA. Dilutions of blood were prepared using filtered, autoclaved, and deionized (FAD) water. The sample substrates used in these experiments included linoleum, glass, metal, painted wood, cloth (65% polyester, 35% cotton; 85% polyester, 15 % cotton; blue denim), and paper (Xerox-grade bond paper; Scott® paper towel). The seven enhancement reagents tested were Amido Black [15,16], Crowle's Double Stain [15,17], Hungarian Red

[15], leucomalachite green [15,18], luminol [6,19], ninhydrin [20], and 1,8-diazafluoren-9-one (DFO) [21]. Each was made up according to recipes used by Royal Canadian Mounted Police (RCMP) Forensic Identification Specialists [2,3].

DNA extraction, quantitation, amplification, and interpretation were done according to RCMP guidelines and procedures [22,23]. See reference 3 for more complete details.

To test the effect of blood enhancement reagents on the quantity of recovered DNA, bloody fingerprints on linoleum were analyzed with and without chemical treatment, using Crowle's Double Stain. In this experiment, 20 µL of blood, ranging in concentration from neat to 1:200, were dropped on to linoleum, allowed to air dry, and then analyzed for the amount of DNA recovered. Bloody fingerprints made using various aliquots and various concentrations of blood were placed on linoleum and allowed to air dry (see Table 1 ). Some were quantitated for DNA without chemical treatment, while others were processed after Crowle's Double Stain had been applied.

To test the effect of blood enhancement chemicals on the DNA profiles recovered, bloodprints were placed on a number of different sample substrates, treated with blood enhancement reagents, and processed for DNA typing. Prints on linoleum, glass, and painted wood were treated with Amido Black, Crowle's Double Stain, Hungarian Red, luminol, and leucomalachite green. Prints on cloth were treated with luminol, and prints on bond paper were treated with ninhydrin and DFO. After treatment, prints were cut out or swabbed, and then analyzed according to the RCMP protocols outlined in Reference 3. As control samples, untreated areas of the substrates were tested, as were areas treated with the blood enhancement reagents alone, and areas where non-bloody fingerprints were applied.

To test the effect of time of enhancement and duration on the DNA results, bloodprints were prepared on a number of surfaces and then treated with enhancement chemicals immediately after drying, or after being left to dry for 7 days or 14 days, then analyzed for DNA. To test the long-term effects of exposure to the enhancement chemicals, fresh and aged bloody fingerprints were treated with enhancement chemicals, then allowed to stand for 7, 14, or 54 days before DNA analysis.

**Results and Discussion**

Most DNA typing protocols require at least I ng of target DNA for successful profiling. In all cases, the 20 µL aliquot of blood, up to dilutions of 1:100, yielded more than sufficient amounts of DNA (see Table 1 and ref. 3). When blood was transferred to a finger to produce a bloody fingerprint, the amount of DNA recovered was understandably reduced. When fingerprints were immediately treated with a blood enhancement reagent such as Crowle's Double Stain, the amount of DNA recovered was reduced by a further 50% (one result indicated a 12-fold decrease in quantity, but this may be due more to an anomalously high yield obtained for the untreated fingerprint). Even then, a fingerprint produced with blood diluted 1:200 and treated with Crowle's Double Stain generated 1.5 ng of DNA. The use of Crowle's Double Stain on non-porous substrates such as linoleum is recognized as one of the most challenging of all seven blood enhancement procedures evaluated. The decrease in DNA yield following enhancement can be attributed to loss of blood cells during the destaining steps carried out in order to reduce the background staining. It is anticipated that other enhancement methods applied to other types of surfaces with different porosity characteristics would promote better DNA yields. These results indicate that significant amounts of DNA can still be recovered after bloodstains have been treated with blood enhancing chemicals. The one potential drawback could be the reduction in the quantity of DNA recovered when specific combinations of blood reagents and surfaces are used.

For all of the surfaces and blood enhancement reagents used here, there were no detrimental effects on the PCR DNA profiles generated. The fluorescent signals and allele size measurements were essentially identical to those of the untreated bloody fingerprints [See ref. 3, Tables 9 and 10]. The only noticeable effect was a strong coloration of the sample during the extraction procedure. While this required a longer purification step, it did not


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have a negative impact on the final DNA result. In the control samples, no quantifiable DNA was recovered, but PCR amplification did result in the appearance of some low intensity signals. For some non-bloody fingerprints applied to glass, almost complete profiles, consistent with the donor of the print, were generated. These results would be in agreement with recent reports of DNA profiles being recovered from epithelial cells left behind when objects are handled [24,25]. The main conclusion from this series of experiments is that the DNA profile generated is not altered by the use of the blood enhancement chemicals, and will not result in an incorrect inclusion or elimination.

Untreated bloodprints left for up to 54 days before DNA analysis produced profiles identical to those generated with fresh samples. Fresh and aged bloodprints subjected to DNA extraction seven, 14, or 54 days after enhancement, generated profiles that showed no allele dropout or additional bands. In a few cases (i.e., Crowle's Double Stain and Hungarian Red), a slight decrease in fluorescent signal was detected across the electrophoretic tracing of the profile, indicating that after 54 days, a slight degradation in the DNA was occurring, resulting in more efficient amplification of smaller STR loci [See ref. 3, Figures 11 and 12]. In other words, bloodstains do not have to be treated immediately with blood enhancing reagents in order to obtain results, and treated bloodstains do not have to be analyzed for DNA immediately after treatment with blood reagents. The one caveat might be that prolonged exposure to some blood reagents applied to certain surfaces can lead to some loss of DNA.

## Conclusions

The results of these experiments indicate that most blood enhancement reagents, commonly used and as tested in our studies, will not have a deleterious effect on subsequent DNA analysis. In all instances, the fluorescent signals were similar and the size measurements of all alleles remained constant and identical to those of the untreated blood. No allele dropout or extraneous bands were detected in profiles generated from the DNA of enhanced bloodprints. Fresh and aged prints enhanced and exposed to reagents for up to 54 days still yielded accurate DNA results. In this respect, Forensic Identification Specialists can confidently use the most commonly employed blood enhancement techniques on bloodstains without concern about compromising subsequent DNA analysis. In only two instances, some degradation of the DNA was observed after 54 days, indicating that prolonged exposure to some blood reagents could eventually lead to less than optimal results.

Our results also indicated that there was some loss of biological material when specific blood enhancement techniques were used. In situations where the amount of blood is small, the loss of blood cells during enhancement may result in insufficient DNA remaining for analysis. Although enhancement does not preclude the obtaining of excellent STR results, it may, when employed on limited samples, have negative consequences and compromise crucial and limited evidentiary samples. It appears that the advancement of DNA technology and blood enhancement detection technologies presents an interesting paradox to the Forensic Identification Specialist. Caution is required when using an enhancement technique on bloodprints to ensure that sufficient biological material is retained by the substrate for possible future DNA submissions. Of course, it is precisely in instances with very little visible blood that enhancement chemistry would be considered and deemed necessary in order to actually define the sample for processing. In some cases, no body fluids are observed prior to enhancement, so swabbing for DNA would not have been contemplated.

## Acknowledgements

We thank Brian Yamashita and Della Wilkinson for many helpful discussions and a careful reading of the manuscript.

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## Table 1

DNA yields from blood drops and bloody fingerprints on linoleum before and after enhancement using Crowle's Double Stain*

| Blood dilution | DNA yield (Total amount of DNA in ng) | | | | | |
|---|---|---|---|---|---|---|
| | Blood Drops | Fingerprint in blood without enhancement | | | | After treatment |
| | 20 µl | 5 µl | 10 µl | 15 µl | 20 µl | 20 µl |
| Undiluted | 1500 | 25 | 175 | 250 | 625 | 315 |
| 1:2 | 250 | — | — | — | — | 80 |
| 1:5 | 80 | — | — | — | — | 40 |
| 1:10 | 50 | 150 | 250 | 125 | 250 | 20 |
| 1:20 | 40 | — | — | — | — | 10 |
| 1:50 | 40 | 2.5 | 2.5 | 1 | 10 | 5 |
| 1:100 | 25 | — | — | — | — | 1.5 |
| 1:200 | — | — | — | — | — | 1.5 |

*This combination of surface and blood enhancement reagent represents the most challenging scenario.