RESEARCH ARTICLE

Effect of Four Latent Blood Visualization Products on DNA

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Abstract

Bloodstain pattern analysts first document visible stains and patterns at a scene. Then, in order to obtain as much information as possible from the scene, they search for latent bloodstains and patterns. Latent bloodstains may be the result of old scenes, scenes altered by weather or fire or clean up attempts to destroy evidence. Due to the existence of false positives, analysts must determine if those latent patterns are blood, human blood and then have DNA analysis performed.

Several chemical products are universally utilized to visualize latent bloodstains. Some have a direct and known negative effect on DNA such as o-tolidine, benzidine and TMB (tetramethylbenzidine) while some others can improve both visualization and DNA analysis after sampling. But how "DNA friendly" they are?

In this article, four different products used to visualize latent bloodstains will be compared. The products tested all have luminol-based formulations that contain varying amounts of hydrogen peroxide. Two of the reagents contain fluorescein.

Introduction

The goal of this experiment is to compare the effect/no effect over time of Bluestar® Forensic, Bluestar® Forensic Magnum, Lumiscene and Lumiscene Ultra on DNA analysis. In order to simulate a crime scene in which blood was subjected to clean-up attempts or otherwise altered, blood dilutions from 1 to 1:2000 were tested. As all analysts use a variety of chemical methods and solution strength to visualize latent bloodstains, three different volumes of products (20, 60 and 100 µl) were added to each dilution. For a statistic goal, three samples were prepared for each volume and dilution. There is usually a delay between the collection of the blood sample and the actual DNA analysis. Therefore, DNA analyses were made at D0, D30 and latter at D60. At least 924 swabs prepared and analyzed.

- D0 = DNA analysis on first day with chemicals in contact with diluted blood
- D30 = DNA analysis at 30 days with chemicals in contact with diluted blood
- D60 = DNA analysis at 60 days with chemicals in contact with diluted blood

In order to complete the study, a second experiment was done to compare two types of immunochromatographic tests Hemoglobin (Hb) test and Rapid Stain Identification of Human Blood (RSID™) were used to confirm the presence of human blood.

The second test revealed human blood without any false positive results. The tests were performed at the Biology department of the Institut de Recherche Criminelle de la Gendarmerie Nationale (IRCGN) in Rosny-Sous-Bois France.

Materials and Methods

Blood Collection

Blood was drawn from a known donor into a collection tube containing EDTA. For all experiments, a total of eight dilutions were done. Dilutions were the following: 1:1, 1:10, 1:20, 1:100, 1:200, 1:500, 1:1000 and 1:2000.

Chemical Reagents for Experiments 1 and 2

Bluestar®
Forensic, Bluestar® Forensic Magnum
Lumiscene
Lumiscene Ultra

Procedure

Experiment 1: DNA Sensitivity

- Each experiment was conducted at D0, D+30 and D+60 periods.
- The samples were prepared in individual Eppendorf® tubes. Three volumes of chemicals were tested (20, 60 and 100µL).
- A volume of 20µL of diluted blood was added to each tube.
- Three swabs were tested for each volume.
- For each period of the experiment, negative and positive controls were performed
- At least 924 swabs were prepared and analysed

The sequential steps are presented in figure 1.

Experiment 2: Human Blood Testing

- Each experiment was performed at D0 and D+60 periods
- For each dilution, one volume of 50µL of chemicals was tested and one volume of 20µL of blood was used.
- For each volume, three spots were put on a mat paper. Each paper was dried at room temperature for 0 and 60 day periods.
- For each period of experiment, some negative and positive controls were performed
- Each sample was prepared by placing the blood volume on a mat followed by the blood chemical volume.
- A total of 328 spots for both tests (2 × 164) were prepared and analyzed for all periods.
- Spots were cut and placed in a specific reagent (specific for the test studied) according to Hemoglobin (Hb) and RSID™ blood testing protocols.
- For each period of experiment, negative and positive controls were performed.

- The sequential steps are presented in Figure 2.

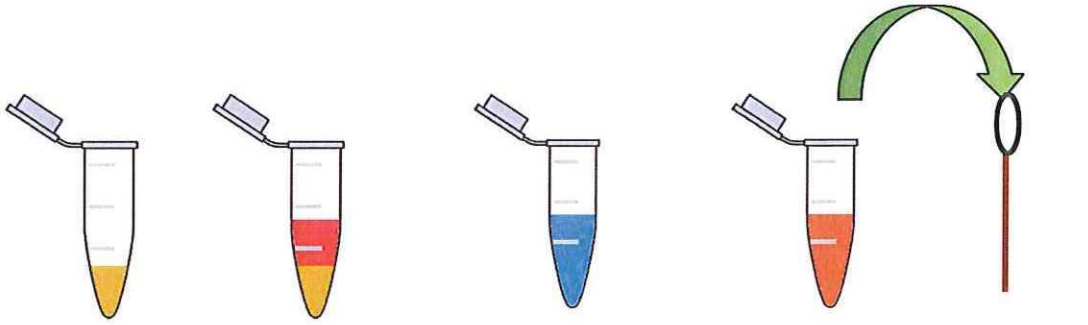

Figure 1. Diagram of sequential steps for experiment 1: DNA sensitivity.

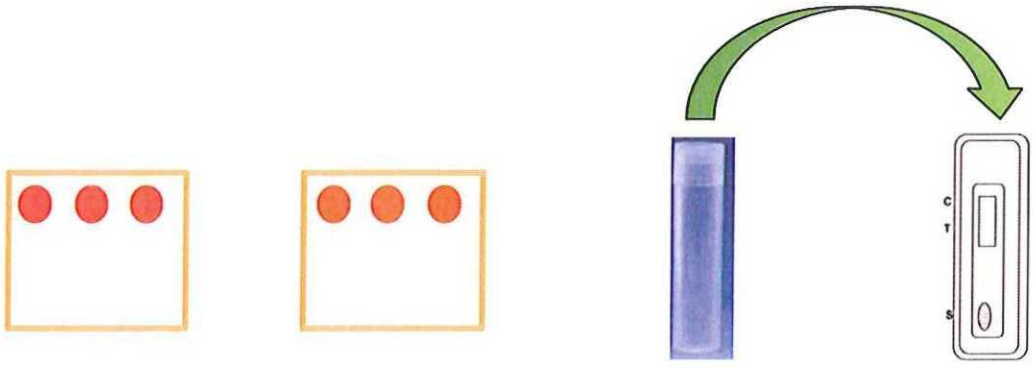

Figure 2. Sequential steps for experiment 2: Human blood testing.

Results

DNA Sensitivity

Each profile was analysed according to their quality based on a score as shown in Table 1.

Table 1. Scoring system for each DNA profile

0	Complete profile (0 or 1 locus affected)
1	Profile with 1 anomaly: Partial or unbalanced (from 2 loci affected)
2	Profile with 2 anomalies: Partial or unbalanced (from 2 loci affected)
3	Non explicable profile

Results at D0

Negative controls did not reveal any positive results and positive controls revealed positive results for all swab from undiluted blood to 1:500 dilution. The results for Lumiscene (L), Bluestar® Forensic (BS) Bluestar® Forensic Magnum (BM) and Lumiscene Ultra (LU) are presented in Tables 2-6.

Table 2. Results for Lumiscene (L)

			Lumiscene							
			Blood dilutions							
			Pure	1/10	1/20	1/100	1/200	1/500	1/1000	1/2000
Chemical volumes (µL)	20	Test 1	0	0	0	0	2	3	2	3
		Test 2	0	0	0	0	1	2	3	3
		Test 3	0	0	0	0	1	2	2	3
	60	Test 1	0	0	0	0	2	3	3	3
		Test 2	0	0	0	0	0	2	3	3
		Test 3	0	0	0	0	2	2	3	3
	100	Test 1	0	0	0	0	2	2	3	3
		Test 2	0	0	0	2	2	2	3	3
		Test 3	0	0	0	0	2	3	3	3

Table 3. Results for Bluestar® Forensic (BS)

			Bluestar							
			Blood dilutions							
			Pure	1/10	1/20	1/100	1/200	1/500	1/1000	1/2000
Chemical volumes (µL)	20	Test 1	0	0	0	1	2	2	2	3
		Test 2	0	0	0	2	1	2	2	3
		Test 3	0	0	0	0	2	2	2	3
	60	Test 1	0	0	0	0	2	2	3	3
		Test 2	0	0	0	0	0	3	3	3
		Test 3	0	0	0	0	1	2	3	3
	100	Test 1	0	0	0	0	2	3	3	3
		Test 2	0	0	0	0	2	2	3	3
		Test 3	0	0	0	0	2	2	2	3

Table 4. Results for Bluestar® Forensic Magnum (BM)

			Bluestar Magnum							
			Blood dilutions							
			Pure	1/10	1/20	1/100	1/200	1/500	1/1000	1/2000
Chemical volumes (µL)	20	Test 1	0	0	0	0	2	2	3	3
		Test 2	0	0	0	0	2	2	3	3
		Test 3	0	0	0	0	2	2	3	3
	60	Test 1	0	0	0	0	2	3	3	3
		Test 2	0	0	0	0	1	3	3	3
		Test 3	0	0	0	1	2	3	3	3
	100	Test 1	0	0	0	2	2	3	3	3
		Test 2	0	0	0	2	2	3	3	3
		Test 3	0	0	0	2	2	3	3	3

Table 5. Results for Lumiscene Ultra (LU)

			Lumiscene Ultra							
			Blood dilutions							
			Pure	1/10	1/20	1/100	1/200	1/500	1/1000	1/2000
Chemical volumes (µL)	20	Test 1	0	0	0	2	2	2	3	3
		Test 2	0	0	0	1	2	3	3	3
		Test 3	0	0	0	2	2	3	3	3
	60	Test 1	0	0	0	2	2	3	3	3
		Test 2	0	0	0	2	3	3	3	3
		Test 3	0	0	1	2	3	3	3	3
	100	Test 1	0	0	0	3	3	3	3	3
		Test 2	0	0	0	3	3	3	3	3
		Test 3	0	0	0	3	3	3	3	3

As demonstrated in tables 2 and 3, Bluestar® Forensic produced better results according to the quality of the DNA profile at the 1:100 dilution than did Lumiscene Ultra. Results were quite similar for the remainder of the dilutions. Bluestar® Forensic did not affect the ability to obtain the known donor's DNA profile at 1:10, 1:20 and 1:100 dilutions of blood. Similar results were obtained with Bluestar® Forensic Magnum with a slight variation at the 1:100 dilutions.

One characteristic of the Lumiscene Ultra chemical is that it is sold only for reconstruction. That is why complete profiles were given only with the 1:10 dilutions. Good results were obtained with a majority of complete profiles for the 1:20 dilutions. Beginning with the 1:100 dilutions, the results showed a strong DNA alteration.

Results at D30

Negative controls did not reveal any positive results and positive controls revealed positive results for swabs from undiluted blood to a 1:500 dilution. The results for Lumiscene (L), Bluestar® Forensic (BS) Bluestar® Forensic Magnum (BM) and Lumiscene Ultra (LU) are presented in Table 6.

Table 6. Results at D30.

		Bluestar			Bluestar Magnum			Lumiscene			Lumiscene Ultra		
		20	60	100	20	60	100	20	60	100	20	60	100
Pure	Test 1	0	0	0	0	0	0	0	0	0	0	0	0
	Test 2	0	0	0	0	0	0	0	0	0	0	0	0
	Test 3	0	0	0	0	0	0	0	0	0	0	0	0
1/10	Test 1	0	0	0	0	0	0	0	0	0	0	0	2
	Test 2	0	0	0	0	0	0	0	0	0	0	0	0
	Test 3	0	0	0	0	0	0	0	0	0	0	1	0
1/20	Test 1	0	0	0	0	0	0	0	0	0	0	0	0
	Test 2	0	0	0	0	0	0	0	0	0	0	0	0
	Test 3	0	0	0	0	0	0	0	0	0	0	0	0
1/100	Test 1	2	2	1	1	0	2	2	1	2	2	2	2
	Test 2	2	0	0	1	1	2	2	0	0	2	2	2
	Test 3	2	1	2	2	2	2	2	0	2	2	0	2
1/200	Test 1	2	2	2	2	2	3	2	2	2	2	2	3
	Test 2	2	2	2	2	3	3	2	2	2	2	2	3
	Test 3	2	2	2	2	3	2	2	2	2	2	2	2

For Lumiscene, from undiluted blood to the 1:20 dilutions, DNA profiles qualities were the same. However, from 1:100 dilutions, light damage. Indeed, there were more scores of 2 than of 0 to the 1:200 dilutions. Scores of 3 appeared at the same dilutions than at D0.

The results for Bluestar® Forensic, as seen in experiments at D0, it is a satisfactory chemical to obtain a DNA profile at 1:10 and 1:20 dilutions. Nevertheless from the 1:100 to the 1:2000 dilutions, results were worse with a majority of scores of 2 for the 1:100 dilutions and a score of 3 for all tests from the 1:500 dilutions. The same trend can be noticed with Bluestar® Forensic Magnum with incomplete profiles obtained from the 1:200 dilutions.

Overall, profiles showed the same quality for Lumiscene Ultra at D0 and D30 with better results at D30 from the 1:100 dilutions with a majority of scores of 2 to the 1:200 dilutions.

Human Blood Identification Tests

Human Blood Testing

Results at D0

Each test was analysed based the on the scoring system as presented in table 7.

Table 7. Scoring system

+++	Positive: very good eye naked line
++	Positive: good eye naked line
+	Positive: eye naked line
+-	Indeterminable
-	Negative

Hb tests

Negatives controls did not reveal any positive results. Positive controls revealed positive results for undiluted blood and for the following dilutions: 1:10, 1:20, and 1:100 and weaker for 1:500 and 1:5000. The results are presented in table 8.

Table 8. Results for Hb test

		HB tests						
		Dilutions						
		Pure	1/10	1/20	1/100	1/500	1/2000	1/5000
BS	Test 1	++	+++	+++	-	-	-	-
	Test 2	++	+++		+	-	-	-
	Test 3	++	+++		-	-	-	-
BM	Test 1	++	+++	+++	+	-	-	-
	Test 2	++	+++		+	-	-	-
	Test 3	++	+++		+-	-	-	-
L	Test 1	++	+++	+++	++	+-	-	-
	Test 2	++	+++		++	+-	-	-
	Test 3	++	+++		+	+-	-	-
LU	Test 1	++	+++	++	+-	-	-	-
	Test 2	++	+++		+-	-	-	-
	Test 3	++	+++		+-	-	-	-

As noted in table 8 positive results were obtained with undiluted and diluted blood (1:10 and 1:20). Results for the 1:100 dilution could be acceptable but they were better with Lumiscene. It was observed that for undiluted blood, results were somewhat worse than for the 1/10 dilution. This could be due to the "Hook Effect" which is a false negative result with certain immunoassays due to very high concentration of a particular reagent or blood.

RSID

Negative controls did not reveal any positive results. Positive controls revealed positive results for undiluted blood and for the 1:10 dilutions as presented in table 9.

Table 9. Results for RSID

		RSID						
		Dilutions						
		Pure	1/10	1/20	1/100	1/500	1/2000	1/5000
BS	Test 1	+++	+	-	-	-	-	-
	Test 2	+++	+		-	-	-	-
	Test 3	+++	+		-	-	-	-
BM	Test 1	+++	++	-	-	-	-	-
	Test 2	+++	++		-	-	-	-
	Test 3	+++	++		-	-	-	-
L	Test 1	+++	+	-	-	-	-	-
	Test 2	+++	+		-	-	-	-
	Test 3	+++	+		-	-	-	-
LU	Test 1	+++	+	-	-	-	-	-
	Test 2	+++	+		-	-	-	-
	Test 3	+++	+		-	-	-	-

Good results were obtained with RSID for undiluted blood and for the 1:10 dilutions as shown in table 9. The difference between these tests and Hb tests may be due to the sought element (hemoglobin for Hb tests and glycophorin A for RSID tests). Detection of glycophorin A seems to be more specific. This can explain why it could be more difficult to obtain a good result with a diluted blood than with haemoglobin detection.

A unique test with the 1:20 blood dilutions was performed for each chemical. Indeed, only the 1:10 and the 1:100 were tested in the first phase. The results for the 1:100 dilutions were significantly lower or absent. That is why it became interesting to test the 1:20 blood dilutions.

Discussion

Lumiscene provided better results for the 1:100 dilutions than the other chemicals. Moreover, most of the results obtained for D0 and D30 show that the quality of genetic profiles are more similar with Lumiscene than with Bluestar®. Genetics profiles are the same for light dilutions for all chemicals.

Additional results for D60 will be obtained soon. Nevertheless, variations according to scores assigned to each profile were noted for D0 and D30. The effect of time was observed. The only variable parameter was the time of contact between blood and chemicals. However, chemicals did not seem to have an effect on time for undiluted blood. Hence, this trend that was observed during one month indicated that the quantity of those products has an effect on the quality of the genetic profiles of diluted blood. This may be due to the period during which chemicals were in contact with blood and/or the quantity of chemicals placed on each swab.

In this experiment, the exact number of leukocytes was unknown. It was not possible to count precisely the number of cells with a specific counter. Blood pipetting was done to allow a homogeneous distribution of cells for each dilution, hence the reason that three samples were tested for each dilution and for each volume of chemicals added.

Only 20 µL of blood dilutions were used on each swab. This parameter may explain some poor results concerning the quality of genetic profiles. We suggest that a larger quantity of blood would provide better genetic profiles.

It is important to underline the fact that no surfaces were tested in this experiment. Bloodstains and patterns are influenced by surface texture and composition upon which they were deposited or come into contact. Indeed, this can have an effect on the absorption, the size and shape of the bloodstains and may also influence the quality of samples.

Conclusions

Results obtained for D0 and D30 seem indicate that Lumiscene is slightly better than Bluestar® Forensic especially at the 1:100 dilutions at the 30 day period. Lumiscene Ultra was tested with the understanding that it is used for reconstruction and not for DNA analysis. Accordingly, poorer results were obtained with a period of one month during which chemicals were in contact with blood. This result appears to be linked with the quantity of chemicals put on swab and the time of contact.

At D0, the quantity of chemicals did not significantly affect the quality of the profile. The tests also revealed that an increase in time and chemical volume more greatly affected the ability to obtain a DNA profile. This trend will be analyzed and perhaps certified with D60 results. With all results, an analysis will be done on 924 data with a statistical study. We hope to publish a scientific article in the near future with all the results and more details will be presented.

With regard to human blood detection, without results for D60, RSID provided worse results than the Hb test despite its specificity. This trend has to be confirmed with experiments at D60 which will be performed in the near future.

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