

# Bluestar<sup>®</sup> Forensic Validation Study

## **I. Equipment and Materials Used**

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## **III. Results of the Genotyping**



Operations Unit for Genetic Imprinting

## I. Equipment and materials used:

### Vaporizer:

- Vaporizer: Supplied in the order of 15 04 2003

### Kit Bluetar :

- Solution test B (1 tablet = 40 mL) supplied by Mr. Lefebvre-Despeaux on 03 10 2003
- Tablets supplied by Mr. Lefebvre-Despeaux on 03 10 2003

## II. Test results:

### 1. Test no 1 : Determination of the sensitivity threshold of the Bluestar kit :

#### 1.1. Record of the manipulation stages:

- Setting up a series of dilutions starting with a pure blood sample preserved on EDTA. This series ranges from the 1/2 dilution sample to the one measuring a 1/1000 dilution; sterile water is used. Testing 20 µL of each solution leads to deposits on a clean and dry cloth. These deposits are subsequently dried in open air. After drying, the series is vaporized with the Bluestar solution in order to be able to effect a visual reading of the luminescence associated with each of the dilutions.
- This series is set up simultaneously by two researchers in two separate locations, using the same sample of pure blood, in order to assure the reproducibility of the manipulation.

**Parameter variant:** The blood concentration deposit on the cloth.

#### 1.2. Results of test n° 1 :

**A genotyping of 16 stains is performed (cf. paragraph III for the results of the genotyping)**

<b>Dilutions 1</b>	<b>1 / 2</b>	<b>1 / 5</b>	<b>1 / 10</b>	<b>1 / 100</b>	<b>1 / 250</b>	<b>1 / 500</b>	<b>1 / 750</b>	<b>1 / 1000</b>
<b>Results</b>	Visible to the naked eye							
<b>Dilutions 2</b>	<b>1 / 2</b>	<b>1 / 5</b>	<b>1 / 10</b>	<b>1 / 100</b>	<b>1 / 250</b>	<b>1 / 500</b>	<b>1 / 750</b>	<b>1 / 1000</b>
<b>Results</b>	Visible to the naked eye							

Photo no 1: Range at daylight. The picture depicts the partition of deposits on the piece of cloth. This picture demonstrates the evolution of the coloration of the samples with reference to blood concentration.



Photo no 2: The Bluestar solution is sprayed and the picture taken immediately afterwards ( $t_0$ ).



### 1.3. Conclusions about test no °1 :

- The photo no 1 allows observation of the sensitivity up to the degree of a 1 / 1 000 solution.
- The results obtained with this new formula are very interesting as they give evidence of a much greater chemo-luminescence intensity and a much longer emission period.
- With these effects, we have used only the naked eye as visual measure in the evaluation of the chemo-luminescence intensity. In this context and with reserve, we may rate the intensity level 1/3 superior to the intensity achieved with the previous formula.
- Moreover, visual detection of the stain lasts at least 10 minutes in the case of pure stains.
- These conclusions will be confirmed in point 3, by the repeatability test.

## 2. Test no°2 : Determination of stains in function of their volume

### 2.1. Record of the manipulation stages:

- Setting up two series of different volume, in order to make distinctions between the macro and the micro stains

**Parameter variant:** The volume of blood deposited.

### 2.2. Results of test no° 2 :

**A genotyping of 8 stains is achieved (a Series stain) (cf. paragraph III for the results of the genotyping)**

<b>Value range 1</b>	<b>6 micro drops deposited separately by deposit zone</b>	<b>3 micro drops superimposed</b>	<b>0,5</b>	<b>1</b>	<b>2,5</b>	<b>5</b>
<b>Results</b>	Visible to the naked eye					
<b>Value range 2</b>	<b>6 micro drops deposited separately by deposit zone</b>	<b>3 micro drops superimposed</b>	<b>0,5</b>	<b>1</b>	<b>2,5</b>	<b>5</b>
<b>Results</b>	Visible to the naked eye					

Photo no 3: Value range in daylight. The picture depicts the deposit traces on the cloth for different volumes of deposits. This picture permits very weak deposits to be seen: in this sample, on a clear cloth, whereas they would remain invisible to the naked eye on a dark cloth background.

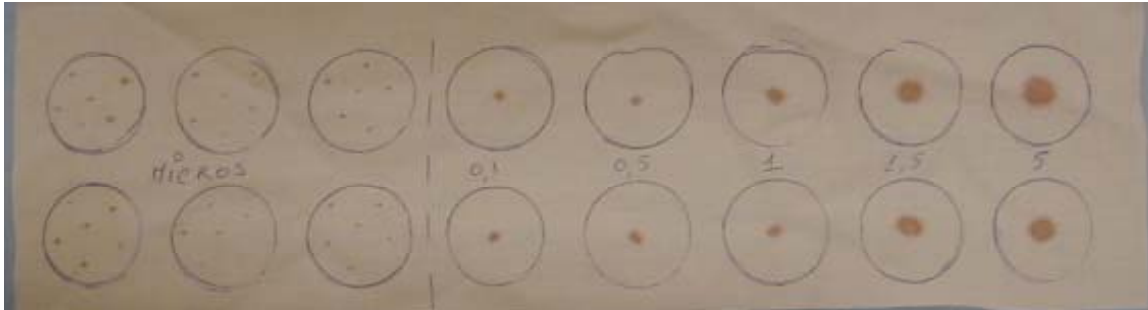
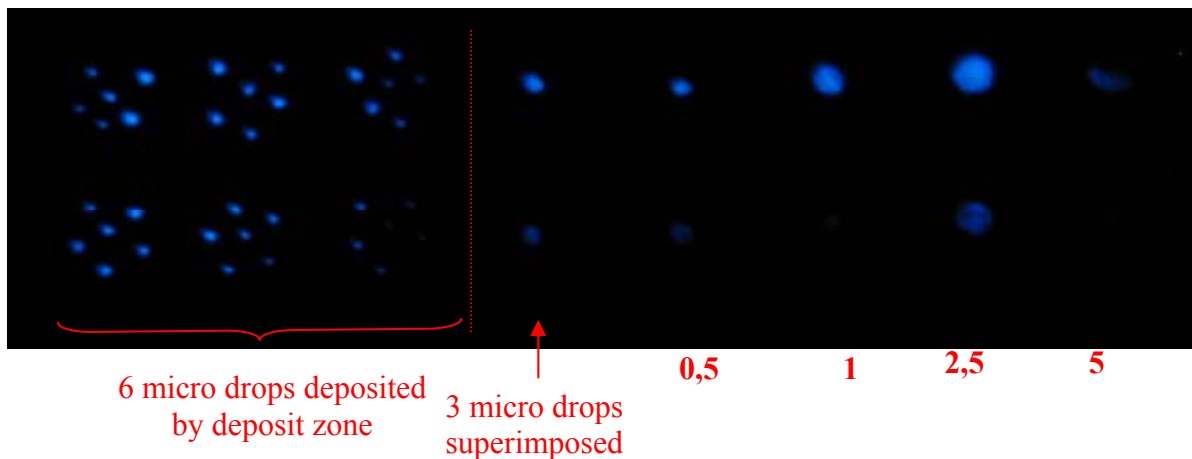


Photo no 4: In the dark, the picture is self-explanatory, as the micro traces are clearly visible and evidence their incontrovertible presence.



### 2.3. Conclusions about test no°2 :

- The visualization of the stains does not depend on their volume but rather on the reaction of the blood with the Bluestar. In fact, with this new formula, the chemo-luminescent reaction being more intense and of longer duration, a more precise study of the various stains is made feasible, which increases the probability of their detection irrespective of the volume of blood deposited (either purposefully or not).

## **3. Test no°3 : Determination of the repeatability of the manipulation**

### 3.1. Record of the manipulations carried out:

- Manipulation of 10 pure blood stains (20  $\mu$ L ) in order to test the repeatability of detection by Bluestar.

**Parameter variant:** Vaporization of Bluestar on the cloth

### 3.2. Results of test no° 3 :

**A genotyping of 10 stains is achieved in order to have a positive control sample (cf. paragraph III for the results of the genotyping)**

Photo no 5: View of the repeatability range in daylight



Photo no 6: View of the repeatability range in the dark



### 3.3. Conclusions:

- After the vaporization of the Bluestar solution on them, the stains give off similar emissions (intensity and chemo-luminescent duration). The intensity level is notably stronger than that of the previous tests carried out with the first formula. This intensity was not evaluated by our own unit but objectively seems to be one third superior to the previous one. With respect to the duration of the chemo-luminescence, we have clearly observed that it could persist on the average for as long as 5 to 10 minutes in case of pure stains. Observations previously made in test 1.
- In order to be able to evaluate the intensity of the luminescence in a more scientific way, it would be interesting to carry out supplementary tests.

## **4. Test no°4 : Determination of detection of blood and deposits of water with bleach**

### 4.1. Record of the manipulations carried out:

- Manipulation of 9 stains (20 µL) of pure blood + same volume of water with bleach
- 1 stain of only water with bleach

**Parameter variant :** The presence of water with bleach

#### 4.2. Results of test no° 4 :

**A genotyping of 10 stains is achieved (cf. paragraph III for the results of the genotyping)**

Photo no°7: View of the test series in the dark



#### 4.3. Conclusions:

- Only the bloodstains were revealed by Bluestar vaporization .
- The water with bleach, which presented a clear false positive but was distinguishable from the blood stains in the previous formula, now appeared so feeble that there could be little doubt in its comparison with the blood stains.
- As a result, the water with bleach keeps giving a false positive representation, but of a totally different amplitude vis-à-vis the previous tests.

### 5. Test no 5: Determination of blood detection following a cleansing with bleach water

#### 5.1. Record of the manipulations carried out:

- Manipulation of 21 stains (20 µL) of pure blood + a volume and a concentration made up of water with bleach
- 3 stains with only water with bleach
- When the bloodstains are dry, spots of water with bleach are deposited. When everything is dry, the cloth is washed in a washing machine and dried. Only after such treatment are the tests on Bluestar carried out.

		(1 :1)	(1 :2)	(1 :3)	(1 :1)	(1 :2)	(1 :2)	Atomization
		bleach at 9.6%			bleach at 0.48 %			
<b>Value range 1</b>	Pure bleach	20 µL Blood + 20 µL bleach	20 µL Blood + 40 µL bleach	20 µL Blood + 60 µL bleach	20 µL Blood + 20 µL bleach	20 µL Blood + 40 µL bleach	20 µL Blood + 60 µL bleach	20 µL Blood + atomiz.°
<b>Value range 2</b>	Pure bleach	20 µL Blood + 20 µL bleach	20 µL Blood + 40 µL bleach	20 µL Blood + 60 µL bleach	20 µL Blood + 20 µL bleach	20 µL Blood + 40 µL bleach	20 µL Blood + 60 µL bleach	20 µL Blood + atomiz.°
<b>Value range 3</b>	Pure bleach	20 µL Blood + 20 µL bleach	20 µL Blood + 40 µL bleach	20 µL Blood + 60 µL bleach	20 µL Blood + 20 µL bleach	20 µL Blood + 40 µL bleach	20 µL Blood + 60 µL bleach	20 µL Blood + atomiz.°

(1 : 1) : 1 volume of Blood + 1 volume of bleach water

(1 : 2) : 1 volume of Blood + 2 volumes of bleach water

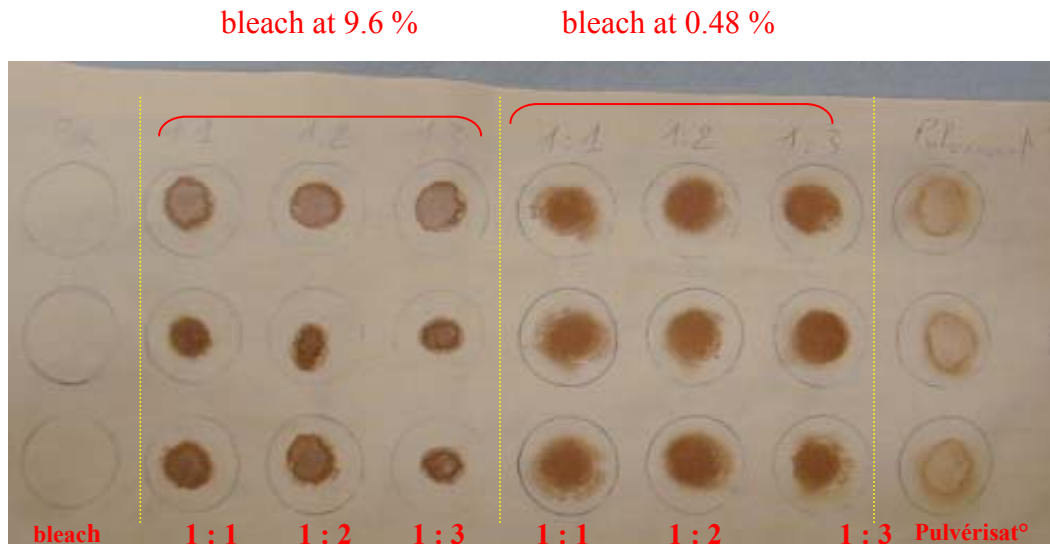
(1 : 3) : 1 volume of Blood + 3 volumes of bleach water

**Parameter variant:** The volume and the concentration of water with bleach

5.2. Results of test no° 5 :

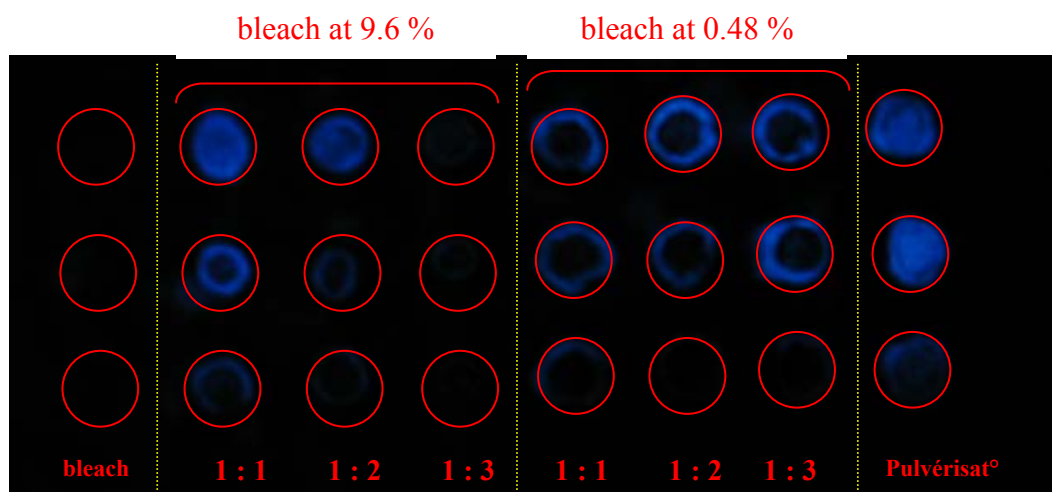
**A genotyping of 24 stains is achieved (cf. paragraph III for the results of the genotyping)**

Photo no 8 : View of the test series using bleach water. This picture depicts the cloth as it appears following washing inside a washing machine (cycle at 40°C – daily cycle with conventional liquid bleach)



These tests were conducted for our laboratory in order to corroborate some previous results. They were not performed specifically to test this formula but they do contribute supplementary elements to the present study.

Photo no 9: View of the test series using bleach water



5.3. Conclusions:

- Photo no 9 clearly shows the absence of false positives to interfere with the reading. In contrast, it is evident that the presence or absence of blood conditions the chemo-luminescence.
- Water with bleach fixes the biological material into the fabric and thus allows for a naked eye view of the red stains produced by the blood following a washing in the washing machine.

### III. Results of the genotyping:

Photo no 1 :

**NSD** : No Size Data

- : the software does not record No Size Data but does not render any useable result

A : Amelogenine

	Designation of the test	Quantifyin g results	Intensity of the A	Number of systems without the A	Comments
	<b>Positive control sample</b>	<b>&gt; 0</b>	2111	10	
1	Repeatability Test: pure blood	> 0	774	10	The 10 systems are useable ; there is no influence by Bluestar on the detection of blood through the genotyping procedure
2	Repeatability Test: pure blood	> 0	771	10	
3	Repeatability Test: pure blood	< 0	2285	10	
4	Repeatability Test : pure blood	> 0	5985	10	
5	Repeatability Test : pure blood	> 0	559	10	
6	Repeatability Test : pure blood	> 0	2331	10	
7	Repeatability Test : pure blood	> 0	80	4	
8	Repeatability Test : pure blood	> 0	798	10	
9	Repeatability Test : pure blood	> 0	1021	10	
10	Repeatability Test : pure blood	> 0	537	10	
11	Blood + bleach (1 : 1)	> 0	1206	10	The 10 systems are useable ; there is no influence by Bluestar mixed with water with bleach. The detection of blood by means of the genotyping procedure has not been put into question by the utilization of this product
12	Blood + bleach (1 : 1)	> 0	669	10	
13	Blood + bleach (1 : 1)	> 0	719	10	
14	Blood + bleach (1 : 1)	> 0	2083	10	
15	Blood + bleach (1 : 1)	> 0	1677	10	
16	Blood + bleach (1 : 1)	> 0	1375	10	
17	Blood + bleach (1 : 1)	> 0	2534	10	
18	Blood + bleach (1 : 1)	> 0	2115	10	
19	Blood + bleach (1 : 1)	> 0	1455	10	
20	bleach on its own	< 0	NSD	NSD	
21	Test volume : micro stain	< 0	193	5	
	<b>Negative control sample</b>		-	-	
23	Test volume : micro stain	< 0	101	2	Only the quantity of the ADN affects the genotyping
24	Test volume : micro stain	< 0	103	1	
25	Test volume : micro stain	< 0	-	1	
26	Test volume : micro stain	< 0	94	2	
27	Test volume : micro stain	< 0	91	2	
28	Test volume : micro stain – 0,1	< 0	268	6	
29	Test volume : micro stain – 0,1	< 0	287	10	



 <p>Est</p>	<b>TEST RESULTS BLUESTAR</b>	<b>1071 EN TEC Version 1</b>
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30	Test volume : micro stain – 0,5	< 0	154	6	
31	Test volume : micro stain – 0,5	< 0	315	9	
32	Test volume : micro stain – 1	< 0	480	10	
33	Test volume : micro stain – 1	< 0	493	10	
34	Test volume : micro stain – 2,5	< 0	1242	10	
35	Test volume : micro stain – 2,5	< 0	721	10	
36	Test volume micro stain – 5	< 0	1546	10	
37	Test volume : micro stain – 5	< 0	NSD	-	
38	<b>Control sample SEB (37)</b>	< 0	NSD	NSD	
39	Dilution test: Pure	> 0	-	-	Only the quantity of the ADN affects the genotyping
40	Dilution test: 1/2	< 0	1453	10	
41	Dilution test: 1/5	< 0	169	5	
42	Dilution test: 1/10	< 0	NSD	NSD	
43	Dilution test: 1/100	< 0	NSD	NSD	
44	<b>Negative control sample</b>		-	-	
45	Dilution test: 1/250	< 0	NSD	NSD	Only the quantity of the ADN affects the genotyping
46	Dilution test: 1/500	< 0	86	2	
47	Dilution test: 1/750	< 0	NSD	NSD	
48	Dilution test : 1/1000	< 0	NSD	NSD	
49	Dilution test : Pure	< 0	1655	10	
50	Dilution test : 1/2	< 0	359	9	
51	Dilution test : 1/5	< 0	255	10	
52	Dilution test : 1/10	< 0	398	9	
53	Dilution test : 1/100	< 0	87	3	Only the quantity of the ADN affects the genotyping
54	Dilution test : 1/250	< 0	-	1	
55	Dilution test : 1/500	< 0	NSD	NSD	
56	Dilution test : 1/750	> 0	NSD	NSD	
57	Dilution test : 1/1000	> 0	NSD	NSD	
58	Test : pure bleach	< 0	89	-	No ADN
59	Test : pure bleach	< 0	NSD	NSD	
60	Test : pure bleach	< 0	-	-	
61	Test bleach : (1 : 1) at 9.6 %	< 0	2757	8	Water mixed with bleach does not affect the detection of the ADN and there are no interactions between it and Bluestar
62	Test bleach : (1 : 1) at 9.6 %	> 0	7170	9	
63	Test bleach : (1 : 1) at 9.6 %	> 0	4572	9	
64	Test bleach : (1 : 2) at 9.6 %	> 0	3012	10	
65	Test bleach : (1 : 2) at 9.6 %	> 0	4025	7	
66	Test bleach : (1 : 2) at 9.6 %	> 0	4697	8	
67	Test bleach : (1 : 3) at 9.6 %	< 0	2107	10	
68	Test bleach : (1 : 3) at 9.6 %	> 0	5519	9	
69	Test bleach : (1 : 3) at 9.6 %	> 0	5642	10	
70	<b>Control sample SEB (68)</b>	< 0	NSD	NSD	
71	Test bleach : (1 : 1) at 0.48 %	< 0	5667	10	Water mixed with

72	Test bleach : (1 : 1) at 0.48 %	> 0	5466	10	bleach does not affect the detection of the ADN and there are no interactions between it and Bluestar
73	Test bleach : (1 : 1) at 0.48 %	> 0	164	10	
74	Test bleach : (1 : 2) at 0.48 %	> 0	6810	10	
75	Test bleach : (1 : 2) at 0.48 %	> 0	7166	10	
76	Test bleach : (1 : 2) at 0.48 %	> 0	7242	10	
77	Test bleach : (1 : 3) at 0.48 %	> 0	5892	10	
78	Test bleach : (1 : 3) at 0.48 %	> 0	7157	10	
79	Test bleach : (1 : 3) at 0.48 %	> 0	7413	10	
80	Test bleach : vaporized at 0.48 %	> 0	4778	10	
81	Test bleach : vaporized at 0.48 %	< 0	3831	9	
82	Test bleach : vaporized at 0.48 %	< 0	2381	9	
83	Negative control sample	< 0	-		

### 1. Conclusion

Bluestar does not affect in any way the detection of the ADN by genotyping.  
Only the absence of ADN (due to dilution) prevents the detection of blood by Bluestar.