FRENCH DEFENSE DEPARTMENT
GENDARMERIE NATIONALE
CRIMINAL RESEARCH INSTITUTE
BIOLOGY DEPARTMENT

The effect of the BlueStar™ blood reagent on DNA typing.

by Aspirant BRENIAUX

CONTENTS

INTRODUCTION

On a presumed crime scene, searching for bloodstains is of critical importance. The morphological, biochemical, and genetic analysis of these stains is fundamental during an investigation.

Consequently, the Thanatology - Anthropology - Odontology (TAO) department of the Criminal Research Institute of the French Gendarmerie Nationale (IRCGN) studies the morphology of such bloodstains. Similarly, specialists from the fingerprints department (EDG) detect the fingerprints contained in these bloodstains. Finally, experts from the biology department (BIO) are tasked with analyzing all the biological evidence, including blood, in order to identify its origin through DNA typing, and later to compare it to reference samples, including those of the defendant or defendants, and of the victim.

When bloodstains are still fresh and large a visual examination may be sufficient to detect them. In practical cases however these stains may be invisible to the naked eye (having been washed or wiped) and it is then necessary to use intensifying chemicals to evidence them.

Two main categories of such chemicals exist for this purpose:

- those who react to the proteins and amino acids contained in the biological fluid (DFO, Amido Black, etc.)
- those who evidence an enzymatic activity (peroxydase activity type): Benzidine, Leucomalachite green (LMG), Leucocrystal violet (LCV), Luminol, BlueStar™, Fluorescein (1)

Some of these chemicals are not currently used anymore due to practical reasons (difficult and time-consuming preparation) or for security reasons (toxicity).

Two studies have been conducted by the IRCGN on the Leucomalachite green (LMG), and the Leucocrystal violet (LCV) chemicals used by the EDG department to evidence bloody fingerprints. The authors have shown that using these chemicals does not allow for DNA typing when blood is found in small quantity (2, 3).

The biology department is therefore interested in the BlueStar™, a new luminol-based chemical, used by the TAO department.

Luminol is a substance that has been known for a long time. It is actually one of the most commonly used chemicals for the detection of bloodstains in crime scenes. The oxidizing of luminol, when mixed with hydrogen peroxide and a catalyst such as the hem nucleus of the hemoglobin (peroxydase activity), emits a chemical luminescence visible in the dark. Therefore, this chemical process evidences blood hemoglobin. However, Quickenden and Cooper have demonstrated that luminol can also produce a chemical luminescence when reacting to some household detergents such as bleach, or to the hemoglobin of animals blood (4, 5). Still, differences in intensity, emission specter, and reaction time between the household cleaners and blood allow for differentiation.

Numerous authors have researched whether treating bloodstains with luminol allows for DNA typing. Miller, for example, shows that it is possible to extract DNA from luminol treated blood after dilution up to 1/200 (6). Similarly, analysis by PCR (Polymerase Chain Reaction) of short tandem repeated sequences (STR) have demonstrated that a luminol treatment did not compromise DNA typing (7, 8, and 9).

BlueStar™ is a luminol based chemical, designed by the ROC IMPORT Company, and used in the hunting fields for the detection of game animals' blood tracks. It is used by the IRCGN because it produces a more intensive and longer lasting chemical luminescence than luminol I and II. The TAO department is using it to analyze the morphology of the bloodstains on crime scenes. The biology department could be interested in its use too, provided it is possible to perform DNA typing after its use.

This study was organized in two successive phases. First, we have attempted to identify a range of pH for which the deterioration of the blood DNA is reduced. During the second phase, we have studied a new formulation of BlueStar™ with an optimized pH based upon the practical results obtained during the first phase. The final objective is to determine if it is possible to obtain DNA typing after treatment.

MATERIALS AND METHODS

1- Creation of the bloodstains

All the experimentation is conducted with fresh blood obtained from the same male donor. Upon drawing, the blood is mixed with an anticoagulant, EDTA, and preserved in test tubes.

Several dilutions of blood are performed, and each experiment is repeated 3 times.

For each dilution, a volume of 10 µl is deposited on a sample Whatman® test paper. The test paper samples have previously been cut under sterile condition in small squares 1,4 cm x 1,4 cm. Each sample is then placed in a plastic sterile tray (Amplitype DNA typing trays, PerkinElmer) in order to reduce the risk of contamination and to avoid material loss due to capillary effect with the support medium.

Upon positioning on the trays, the bloodstains are allowed to dry at room temperature for 4 hours.

2- Treatment of the bloodstains with soda and BlueStar™

A volume of 15 µl of sodium hydroxyde (NaOH) in incremental concentrations and of BlueStar™, is prepared at the time of test and deposited on each bloodstain sample with a precision pipette making sure that the solution covers the entire bloodstain.

The bloodstains are then subjected to treatment at room temperature for different periods of time: 24 hours, 48 hours, 7 days, 15 days, and 30 days, as well as 4 hours and 12 hours only for a NaOH treatment.

In the mean time, untreated bloodstains are analyzed for reference.

2- Organic extraction of the DNA

Organic extraction (phenol-chloroform method) is conducted in accordance with the test method used by the biology department (ME78).

a- Digestion by proteinase K :

The bloodstains are incubated overnight at 56°C in an enzymatic digestion compound that contains:

- 400 µl of SEB compound
- 10 µl of proteinase K at 10 mg/ml (MERCK)
- 16 µl of DTT 1M (Sigma)

The next morning, the samples are mixed and drained by centrifuging at 12,900 g for 3 minutes.

b- Organic extraction:

- Addition of 500 µl of Isoamylic Phenol-Chloroform-Isoamyl Alcohol (25:24:1), pH 8, Tris 10 mM, EDTA 1 mM (Sigma)
- Shaking for 10 minutes at room temperature
- Centrifuging for 5 minutes at 12,000 g
- Removal of the aqueous upper layer
- Addition of 500 µl of Chloroform (Sigma)
- Shaking for 10 minutes at room temperature
- Centrifuging for 5 minutes at 12,000 g
- Removal of the aqueous layer
- Mixing of a solution of 240 µl of NaCl 5 M (Sigma) and 30 µl of glycogen (ROCHE)
- Addition of 9 µl of this solution to each sample
- Shaking for a few seconds
- Addition of 1 ml of frozen absolute ethanol (Carlo Erba) to each sample
- Shaking for a few seconds
- Freezing for 4 hours at - 20°C to allow for DNA precipitation
- Centrifuging for 30 minutes at 4°C and 12,900 g
- Elimination of the upper layer
- Washing of the deposit in 1 ml of freezing 70% ethanol (Carlo Erba)
- Centrifuging for 15 minutes at 4°C and 12,900 g
- Elimination of the upper layer
- Vacuum drying of the deposits for 10 minutes at 60°C
- Soaking of the deposits in 60 µl of sterile water
- Incubation for 2 hours at 56°C
- Storage at - 20°C

4- Quantification of the DNA

In order to determine the DNA amount, the DNA is quantified using the "Dot Blot" method commonly used in forensic science. The quantification of human DNA is done using the protocol specified in the Kit "ACES™ 2.0+ Human DNA Quantification System" (Life Technologies).

The operating principle consists in the hybridization of an oligonucleotide probe D1721 with DNA extracts captured on a nylon membrane. This probe, specific to human DNA, recognizes a centromeric area of chromosome 17. Adding an enzyme to the probe allows for detection by chemical luminescence.

In order to perform typing, the DNA is quantified by spectrofluorimetry. To conduct this quantification, 20 µl of DNA is added to 180 µl of a Picogreen® solution. Each sample is then placed in a well in a 96-wells plate. It is then analyzed by spectrofluorimeter.

The same quantification process is repeated in parallel on 15 different samples of incremental concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 5, 10 nanogram by microliter).

5- STR typing of the DNA

PCR (Polymerase Chain Reaction) is an enzymatic process that allows, starting with very small quantities of DNA, to amplify the fragments defined by two primers framing the area of DNA to study.

This technique uses the biochemical characteristics of the Taq polymerase: an enzyme capable of synthesizing a complementary strain of DNA from a single strain DNA matrix and a primer. The PCR reaction is made of a succession of cycles that include 3 phases: a denaturation phase, a annealing phase, and a phase of DNA synthesis.

During this process, short sequences repeated in tandem are amplified by PCR according to the protocol described in the Kit SGM+ (Applied Biosystems).

PCR is accomplished by coamplification of 11 loci.

Upon completion of this phase, the fragments of amplified DNA are separated by electrophoresis in a capillary (sequencer ABI 310 Applied Biosystems). Fluorescent markers coupled to the primers allow for the identification of the different loci.

RESULTS

This study has two objectives. First, a range of pH (basic) must be identified wherein the DNA is not, or little, degraded. Then, a solution of BlueStar™ will be prepared in the range of desirable pH, as determined by the results previously obtained, and it will be assessed whether DNA typing is possible after treatment of the bloodstains by this solution of BlueStar™.

1- Determination of the pH of the solution

To study the effect of the pH on the DNA, different solutions of NaOH are tested at the following pH:

- pH = 10
- pH = 10.5
- pH = 11
- pH = 11.5
- pH =12
- pH =12.5
- pH =13
- Sterile water

Eight blood dilutions are produced:

- 0 = sterile water
- 1 = pure blood
- 1/50
- 1 /100
- 1/500
- 1/1,000
- 1 /5,000
- 1/10,000

The preparation, then the processing of the bloodstains is conducted in the same manner as indicated in the chapter “Materials and Methods”.
The quantification is conducted using the “Dot Blot” technique.
For lack of time, and due to technical problems, the DNA of the samples treated for 7 days, 15 days, and 30 days could not be quantified. Nonetheless, results were obtained for the samples treated for 4 hours, 12 hours, 24 hours, and 48 hours (see tabulations 1, 2, 3, and 4).
The results from the samples treated for 4 hours and 12 hours do not evidence a range of pH deteriorating the DNA. In fact, the quantification shows the presence of DNA even when pH = 13. However, in the samples treated for 24 hours and 48 hours, there is no DNA in the samples treated with a soda solution with a pH above 11.5.

In summary, a pH above 11.5 starts degrading the DNA after 24 hours, and this alteration is confirmed after 48 hours.

The pH of the solution of BlueStar™ must then be maintained between 10 and 11.5.

2- DNA typing after treatment of the bloodstains with BlueStar™

Subsequent to the above determination, the ROC IMPORT Company designed a new formulation of BlueStar™ that has a final solution pH of 11.4, measured after dissolution.

The purpose of the second phase of this study is then to determine if DNA typing can be performed on bloodstains treated with this new solution of BlueStar™.

Using the same process as previously described, blood dilutions are prepared:

- Pure blood (not diluted): 1
- 1/10
- 1/50
- 1/100
- 1/1,000
- Negative extraction reference (sterile water)

The bloodstains are treated with this new solution of BlueStar™, prepared with a tablet of H_2O_2 (hydrogen peroxide) added to a volume of 170 ml of solution. The DNA is extracted using the organic method, then quantified by spectrofluorimetry. The purpose of the quantification is to determine the optimal quantity of DNA to be amplified by PCR. Because of the limitations of the spectrofluorimeter (unreliable results under 0.5 ng/µl), 20 µl of the samples with a concentration lower than 0.5 nanogram by microliter (ng/µl) is used for the PCR. On the contrary, samples with a concentration higher than 0.5 ng/µl are diluted so that the final concentration for the PCR be 0.1 ng/µl. Then, 1 µl of amplified DNA is used for typing.

The results of the typing lead to three important findings (see tabulations 5, 6, 7, and 8) :

1- It is not possible to perform DNA typing when blood is diluted at 1/1,000, even when the blood is not treated.

2- DNA typing performed on blood treated with BlueStar™ yields the same results as obtained from typing non treated blood (see graphs 1 and 2).
The frequency, for each profile, indicates the probability of finding the same profile in the general population. It is apparent that the frequencies are virtually identical between the two samples, one treated with BlueStar™, and the other not treated.
Such results are extremely important in forensic science. Actually, obtaining reliable DNA typing (high frequency), even partial, allows for a case determination with a minimal risk of error.

3- Finally, the two findings listed above are still valid for blood treated for 30 days with BlueStar™ (see graphs 3).

CONCLUSIONS AND PERSPECTIVES

Determining in a first stage an ideal basic pH that does not degrade DNA, allowed the ROC IMPORT Company to design a blood reagent solution that could be useful in forensic science. This new solution of the BlueStar™, with a pH of 11.4, offers numerous advantages over a classic solution of luminol. The luminescence intensity is higher, it lasts longer, and it does not necessitate total darkness to be visible. Further, this chemical is more stable in time, and can still be used 30 days after preparation.

This higher reactivity could lead to concerns about a possible degradation of the DNA when the solution is applied on bloodstains.

Actually, this study clearly demonstrates that a treatment with this new solution of BlueStar™ does not prevent reliable DNA typing (high frequency). It is even possible to type blood diluted at 1/100 (theoretical quantity: 0.03 ng/µl) after treatment for 30 days. These results are very encouraging, and make this chemical an interesting tool for forensic science.

However, further studies are necessary to test this product more thoroughly. An experiment on treatment for more than 30 days would be useful. Intermediary blood dilutions between 1/100 and 1/1,000 should also be studied to determine the minimum quantity of DNA necessary for typing. Further, using a more sensitive PCR kit (currently available) would allow for just as reliable results from lower concentrations. Finally, a study should be conducted using other different support mediums in order to broaden the application to a maximum number of different situations as may be encountered on crime scenes.

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ANNEXES

pH of the soda / Blood dilution	7	10	10,5	11	11,5	12	12,5	13	Base reference	
0				< 0,4		<0,2			40	0,1
1	2	0,4	4	0,4	3	4	4	10	20	0,04
1/50	0,2	0,2	0,2	0,2		0,2			10	N-
1/100		<0,2	<0,2	<0,2	<0,2	<0,2			4	
1/500		<0,2							2	
1/1000									1	
1/5000				0,2					0,4	
1/10000									0,2	

Table 1 : QUANTITY OF DNA AFTER TREATMENT WITH A SODA SOLUTION FOR 4 HOURS
Results are expressed in nanogram by microliter (ng/µl) N- = Negative reference (-) : No DNA

pH of the soda / Blood dilution	7	10	10,5	11	11,5	12	12,5	13	Base reference	
0	—								40	0,1
1	<10	10	4	5	10	4	<10	10	20	0,04
1/50	0,4	1	0,2	0,2	0,2	0,2	0,3	1	10	N-
1/100	0,1	0,1	0,1	0,1	0,1	0,1	0,2	0,2	4	
1/500	—	—	—	—	—	—	—	—	2	
1/1000	—	—	—	—	—	—	—	—	1	
1/5000	—	—	—	—	—	—	—	—	0,4	
1/10000	—	—	—	—	—	—	—	—	0,2	

Table 2 : QUANTITY OF DNA AFTER TREATMENT WITH A SODA SOLUTION FOR 12 HOURS
Results are expressed in nanogram by microliter (ng/µl) N- = Negative reference (-) : No DNA

pH of the soda / Blood dilution	7	10	10,5	11	11,5	12	12,5	13	Base reference	
0	—								40	0,1
1	10	10	1	1	1	1	0,4	1	20	0,04
1/50	0,04	0,1	0,1	0,1	0,2	—	—	—	10	N-
1/100	—	—	0,04	0,04	0,1	—	—	—	4	
1/500	—	—	—	—	—	—	—	—	2	
1/1000	—	—	—	—	—	—	—	—	1	
1/5000	—	—	—	—	—	—	—	—	0,4	
1/10000	—	—	—	—	—	—	—	—	0,2	

Table 3 : QUANTITY OF DNA AFTER TREATMENT WITH A SODA SOLUTION FOR 24 HOURS
Results are expressed in nanogram by microliter (ng/µl) N- = Negative reference (-) : No DNA

pH of the soda / Blood dilution	7	10	10,5	11	11,5	12	12,5	13	Base reference	
0	—								40	0,1
1	10	10	10	10	10	10	<4	0,4	20	0,04
1/50	0,1	0,2	0,2	0,2	0,2	0,1	0,2	—	10	N-
1/100	—	0,2	0,1	0,1	0,1	0,1	<0,1	0,04	4	
1/500	—	—	—	—	—	—	—	—	2	
1/1000	—	—	—	—	—	—	—	—	1	
1/5000	—	—	—	—	—	—	—	—	0,4	
1/10000	—	—	—	—	—	—	—	—	0,2	

Table 4 : QUANTITY OF DNA AFTER TREATMENT WITH A SODA SOLUTION FOR 48 HOURS
Results are expressed in nanogram by microliter (ng/µl) N- = Negative reference (-) : No NA

TABLE 5 – Treatment with BlueStar™ for 24 hours												
DNA Type of each sample and its frequency												
LOCI	**NON TREATED SAMPLES**						**SAMPLES TREATED WITH BLUESTAR™**					
Blood dilutions	**0**	**1**	**1/10**	**1/50**	**1/100**	**1/1000**	**0**	**1**	**1/10**	**1/50 (4)**	**1/100**	**1/1000**
D3S1358	Negative extraction reference: no DNA	15 - 16	15 - 16	15 - 16	15 - 16	15	Negative extraction reference: no DNA	15 - 16	15 - 16 (1)	15	15 - 16 (1)	ND
vWA		19 - 19	19 - 19	19 - 19	19 - 19	19		19 - 19	19 - 19	19 - 19	19 - 19	ND
D16S539		11 - 12	11 - 12	11 - 12	11 - 12	ND		11 - 12	11 - 12	11 (2)	11 - 12	ND
D2S1338		18 - 25	18 - 25	18 - 25	18 - 25	ND		18 - 25	18 - 25	ND	18 - 25	ND
Amelogenin		X - Y	X - Y	X - Y	X - Y	X - Y		X - Y	X - Y	X - Y	X - Y	X - Y
D8S1179		9 - 10	9 - 10	9 - 10	9 - 10	9 - 10		9 - 10	9 - 10	9 - 10	9 - 10	ND
D21S11		29 - 29	29 - 29	29 - 29	29 - 29	ND		29 - 29	29 - 29	29 – 33 (3)	29 - 29	29 - 29
D18S51		15 - 16	15 - 16	15 - 16	15 - 16	ND		15 - 16	15 - 16	14 (3)	15 - 16	ND
D19S433		14 - 14	14 - 14	14 - 14	14 - 14	14 - 14		14 - 14	14 - 14	14 - 14	14 - 14	ND
THO1		9 - 9.3	9 - 9.3	9 - 9.3	9 - 9.3	ND		9 - 9.3	9 - 9.3	9 (2)	9 - 9.3	ND
FGA		19 - 20	19 - 20	19 - 20	19 - 20	ND		19 - 20	19 - 20	ND	19 - 20	ND
1/Frequency		8.20437E+14				47,977.57		8.20437E+14		47,490,286	8.2E+14	24.02922

1/Frequency = Probability of finding the same profile in the general population.
ND = Non Determined

(19 - 20) = Alleles present at the locus level. If two alleles are different = heterozygous. If they are the same = homozygous.

Note: All fluorescence peaks with intensity > 75 RFU were selected.

(1) Majority of 15 - 16 profiles. Presence of 2 minor and non significant artefact peaks (14 - 17). (Stochastic effect).
(2) Partial profile
(3) Artefact peak
(4) The difference between treated and non treated 1/50 samples is not due to the action of with BlueStar™ but to the initial quantity of biological material. This statement is confirmed by the fact that the 1/100 treated sample yields a profile identical to that of the non-treated sample.

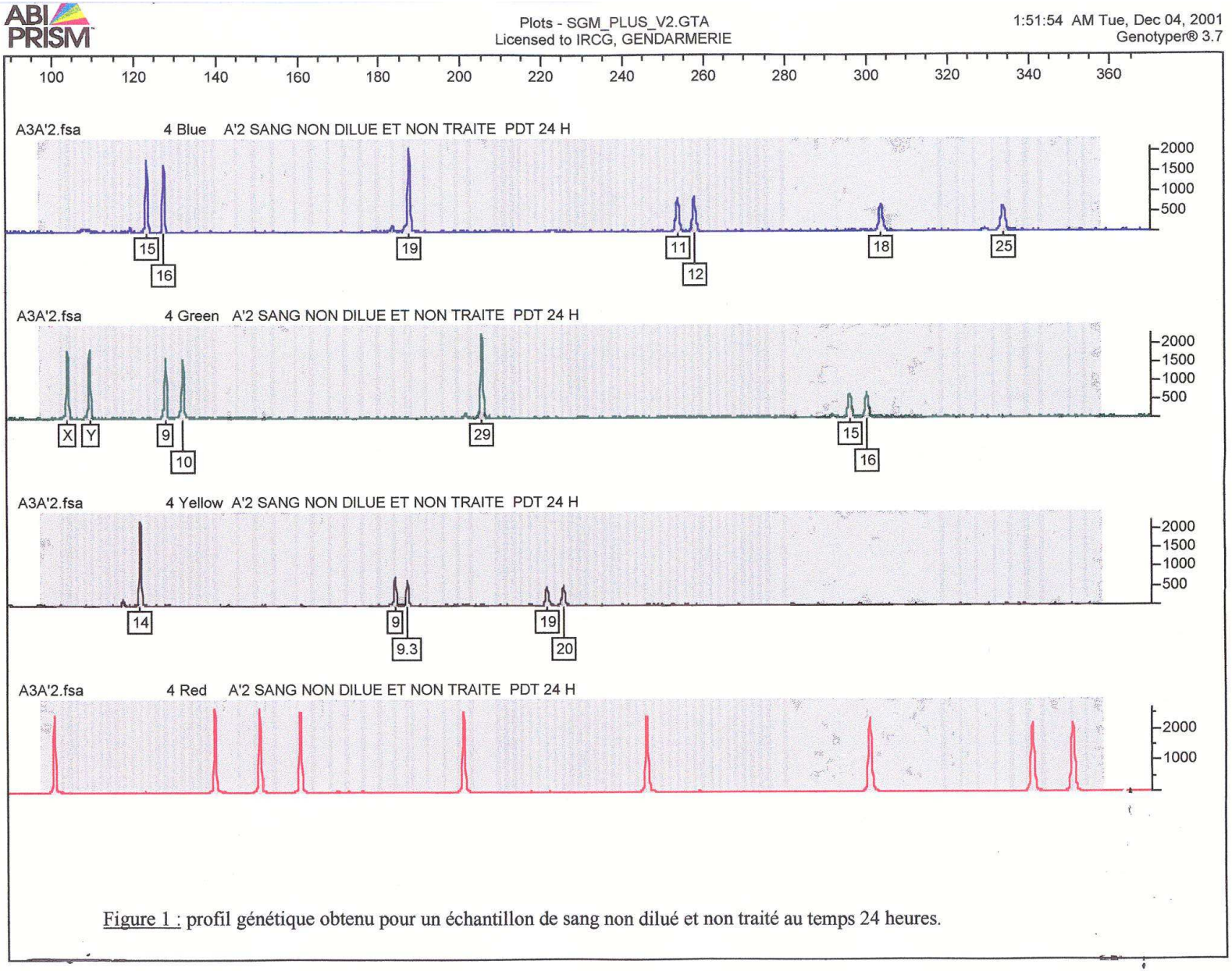

Figure 1 : profil génétique obtenu pour un échantillon de sang non dilué et non traité au temps 24 heures.

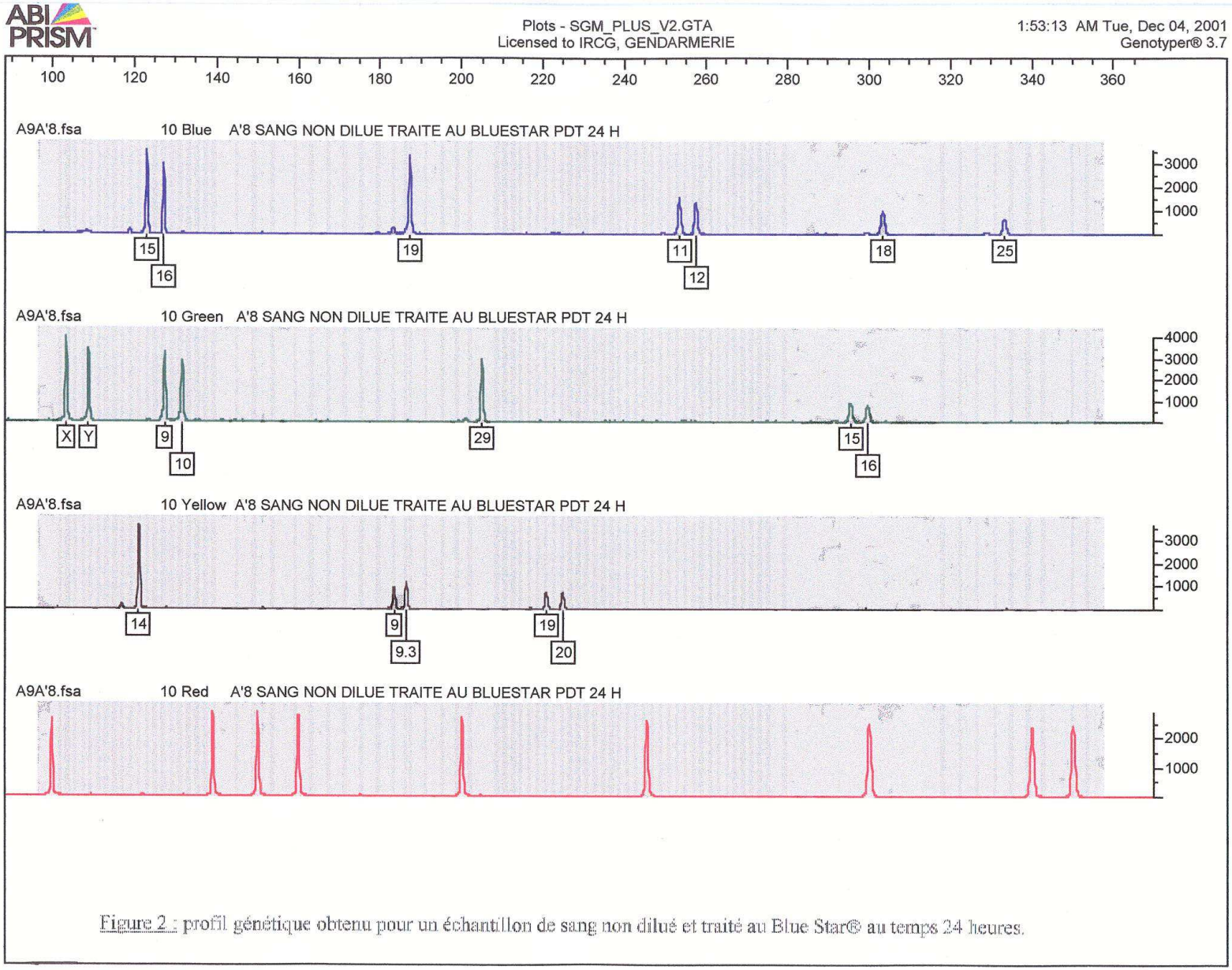

Figure 2 : profil génétique obtenu pour un échantillon de sang non dilué et traité au Blue Star® au temps 24 heures.

TABLE 6 – Treatment with BlueStar™ for 48 hours												
DNA Type of each sample and its frequency												
LOCI	**NON TREATED SAMPLES**						**SAMPLES TREATED WITH BLUESTAR™**					
Blood dilutions	**0**	**1**	**1/10**	**1/50**	**1/100**	**1/1000**	**0**	**1**	**1/10**	**1/50 (4)**	**1/100**	**1/1000**
D3S1358	Negative extraction reference: no DNA	15 - 16	15 - 16	15 - 16	15 (1)	ND	Negative extraction reference: no DNA	15 - 16	15 - 16	15	15 - 16 - 17 (2)	ND
vWA		19 - 19	19 - 19	19 - 19	19 - 19	19		19 - 19	19 - 19	19 - 19	18 - 19	ND
D16S539		11 - 12	11 - 12	11 - 12	11 - 12	ND		11 - 12	11 - 12	11 - 12	11 - 12	ND
D2S1338		18 - 25	18 - 25	18 - 25	18 - 25	ND		18 - 25	18 - 25	18 - 25	18 - 25	ND
Amelogenin		X - Y	X - Y	X - Y	X - Y	X - Y		X - Y	X - Y	X - Y	X - Y	ND
D8S1179		9 - 10	9 - 10	9 - 10	9 - 10	ND		9 - 10	9 - 10	9 - 10	9 - 10	ND
D21S11		29 - 29	29 - 29	29 - 29	29 - 29	ND		29 - 29	29 - 29	29 - 29	29 - 29	ND
D18S51		15 - 16	15 - 16	15 - 16	15 - 16	ND		15 - 16	15 - 16	15 - 16	15 (1)	ND
D19S433		14 - 14	14 - 14	14 - 14	14 - 14	ND		14 - 14	14 - 14	14 - 14	14 - 14	ND
THO1		9 - 9.3	9 - 9.3	9 - 9.3	9 - 9.3	ND		9 - 9.3	9 - 9.3	9 - 9.3	9 - 9.3	ND
FGA		19 - 20	19 - 20	19 - 20	ND	ND		19 - 20	19 - 20	19 - 20	ND	ND
1/Frequency:		8.20437E+14			3.16E+12	145.1589		8.20437E+14			4.3E+11	1

1/Frequency = Probability of finding the same profile in the general population.
ND = Non-Determined
(19 - 20) = Alleles present at the locus level. If two alleles are different = heterozygous. If they are the same = homozygous.

Note: All fluorescence peaks with intensity > 75 RFU were selected.

(1) Partial profile due to small quantity of biological material
(2) Artefact peak

TABLE 7 – Treatment with BlueStar™ for 7 days												
DNA Type of each sample and its frequency												
LOCI	**NON TREATED SAMPLES**						**SAMPLES TREATED WITH BLUESTAR™**					
Blood dilutions	**0**	**1**	**1/10**	**1/50**	**1/100**	**1/1000**	**0**	**1**	**1/10**	**1/50 (4)**	**1/100**	**1/1000**
D3S1358	Negative extraction reference: no DNA	15 - 16	15 - 16	15 - 16	15 - 16	15	Negative extraction reference: no DNA	15 - 16	15 - 16	15 - 16	15 (1)	ND
vWA		19 - 19	19 - 19	19 - 19	19 - 19	ND		19 - 19	19 - 19	19 - 19	19 - 19	ND
D16S539		11 - 12	11 - 12	11 - 12	11 - 12	ND		11 - 12	11 - 12	11 - 12	12 (1)	ND
D2S1338		18 - 25	18 - 25	18 - 25	18 - 25	18		18 - 25	18 - 25	18 - 25	25 (1)	ND
Amelogenin		X - Y	X - Y	X - Y	X - Y	ND		X - Y	X - Y	X - Y	X - Y	X - Y
D8S1179		9 - 10	9 - 10	9 - 10	9 - 10	ND		9 - 10	9 - 10	9 - 10	9 - 10	9
D21S11		29 - 29	29 - 29	29 - 29	29 - 29	ND		29 - 29	29 - 29	29 - 29	29 - 29	ND
D18S51		15 - 16	15 - 16	15 - 16	15 - 16	ND		15 - 16	15 - 16	15 - 16	ND	ND
D19S433		14 - 14	14 - 14	14 - 14	14 - 14	ND		14 - 14	14 - 14	14 - 14	14 - 14	ND
THO1		9 - 9.3	9 - 9.3	9 - 9.3	9 - 9.3	ND		9 - 9.3	9 - 9.3	9 - 9.3	9 - 9.3	ND
FGA		19 - 20	19 - 20	19 - 20	19 - 20	ND		19 - 20	19 - 20	19 - 20	19 - 20	ND
1/Frequency:		8.20437E+14				15.684798		8.20437E+14			1.385E+11	45.454545

1/Frequency = Probability of finding the same profile in the general population.
ND = Non Determined
(19 - 20) = Alleles present at the locus level. If two alleles are different = heterozygous. If they are the same = homozygous.

Note: All fluorescence peaks with intensity > 75 RFU were selected.

(1) Partial profile due to the presence of deteriorated DNA or in too small quantity.

TABLE 8 – Treatment with BlueStar™ for 15 days												
DNA Type of each sample and its frequency												
LOCI	**NON TREATED SAMPLES**						**SAMPLES TREATED WITH BLUESTAR™**					
Blood dilutions	**0**	**1**	**1/10**	**1/50**	**1/100**	**1/1000**	**0**	**1**	**1/10**	**1/50 (4)**	**1/100**	**1/1000**
D3S1358	Negative extraction reference: no DNA	15 - 16	15 - 16	15 (1)	15 (1)	ND	Negative extraction reference: no DNA	15 - 16	15 - 16	15 - 16	15 - 16	ND
vWA		19 - 19	19 - 19	19 - 19	19 - 19	ND		19 - 19	19 - 19	19 - 19	19 - 19	ND
D16S539		11 - 12	11 - 12	11 - 12	11 - 12	ND		11 - 12	11 - 12	11 - 12	11 - 12	ND
D2S1338		18 - 25	18 - 25	18 - 25	18 - 25	ND		18 - 25	18 - 25	18 - 25	(1)	ND
Amelogenin		X - Y	X - Y	X - Y	X - Y	ND		X - Y	X - Y	X - Y	X - Y	ND
D8S1179		9 - 10	9 - 10	9 - 10	9 - 10	ND		9 - 10	9 - 10	9 - 10	9 - 10	ND
D21S11		29 - 29	29 - 29	29 - 29	29 - 29	ND		29 - 29	29 - 29	29 - 29	29 - 29	ND
D18S51		15 - 16	15 - 16	15 - 16	(1)	ND		15 - 16	15 - 16	15 - 16	(1)	ND
D19S433		14 - 14	14 - 14	14 - 14	14 - 14	ND		14 - 14	14 - 14	14 - 14	14 - 14	ND
THO1		9 - 9.3	9 - 9.3	9 - 9.3	9 - 9.3	ND		9 - 9.3	9 - 9.3	9 - 9.3	9 - 9.3	ND
FGA		19 - 20	19 - 20	19 - 20	19 - 20	ND		19 - 20	19 - 20	19 - 20	(1)	ND
1/Frequency:		8.20437E+14			1.56E+09	1		8.20437E+14			6.874E+09	1

1/Frequency = Probability of finding the same profile in the general population.
ND = Non Determined
(19 - 20) = Alleles present at the locus level. If two alleles are different = heterozygous. If they are the same = homozygous.

Note: All fluorescence peaks with intensity > 75 RFU were selected.

(1) Partial profile due to the presence of deteriorated DNA or in too small quantity.

TABLE 9 – Treatment with BlueStar™ for 30 days												
DNA Type of each sample and its frequency												
LOCI	**NON TREATED SAMPLES**						**SAMPLES TREATED WITH BLUESTAR™**					
Blood dilutions	**0**	**1**	**1/10**	**1/50**	**1/100**	**1/1000**	**0**	**1**	**1/10**	**1/50 (4)**	**1/100**	**1/1000**
D3S1358	Negative extraction reference: no DNA	15 - 16	ND	ND	15	ND	Negative extraction reference: no DNA	15 - 16	15 - 16	ND	15 - 16	ND
vWA		19 - 19	ND	19 - 19	19 - 19	ND		19 - 19	19 - 19	19 - 19	19 - 19	ND
D16S539		11 - 12	ND	11 - 12	11	ND		11 - 12	11 - 12	11 - 12	11 - 12	12
D2S1338		18 - 25	ND	ND	18	ND		18 - 25	18 - 25	18 - 25	18 - 25	ND
Amelogenin		X - Y	X - Y	X - Y	X - Y	ND		X - Y	X - Y	X - Y	X - Y	ND
D8S1179		9 - 10	9	9 - 10	9 - 10	ND		9 - 10	9 - 10	9 - 10	9 - 10	ND
D21S11		29 - 29	ND	29 - 35	29 - 29	ND		29 - 29	29 - 29	29 - 29	29 - 29	ND
D18S51		15 - 16	ND	ND	ND	ND		15 - 16	15 - 16	15 - 16	15 - 16	ND
D19S433		14 - 14	ND	14 - 14	14 - 14	ND		14 - 14	14 - 14	14 - 14	14 - 14	ND
THO1		9 - 9.3	ND	ND	9 - 9.3	ND		9 - 9.3	9 - 9.3	9 - 9.3	9 - 9.3	ND
FGA		19 - 20	ND	ND	19 - 20	ND		19 - 20	19 - 20	ND	19 - 20	ND
1/Frequency:		3.378E+22	45.454545	7,441,369.3	2.205E+11	1		8.20437E+14		5.912E+10	8.20437E+14	1.6556291

1/Frequency = Probability of finding the same profile in the general population.
ND = Non Determined
(19 - 20) = Alleles present at the locus level. If two alleles are different = heterozygous. If they are the same = homozygous.

Note: All fluorescence peaks with intensity > 75 RFU were selected. Differences are only related to samples.

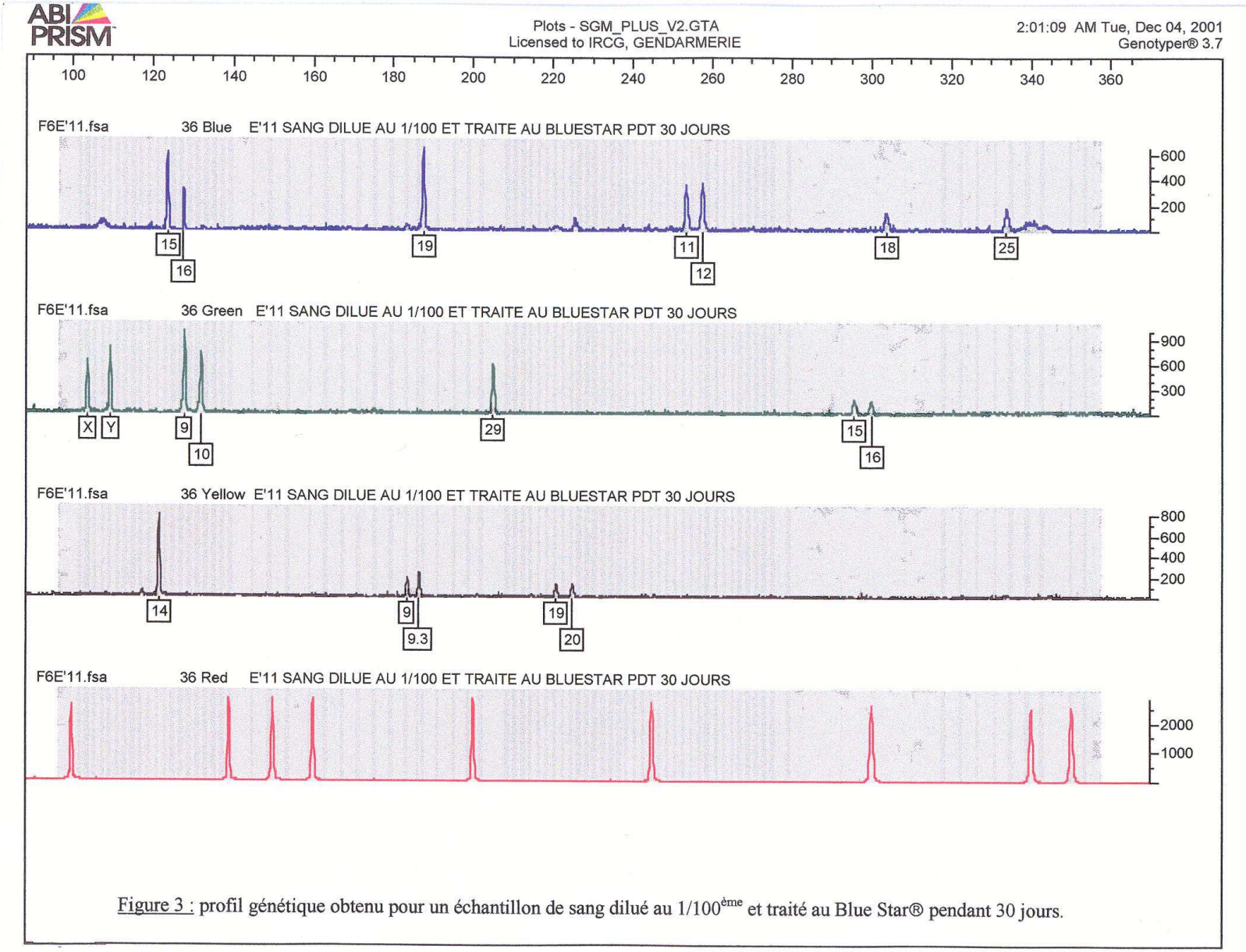

Figure 3 : profil génétique obtenu pour un échantillon de sang dilué au 1/100ème et traité au Blue Star® pendant 30 jours.