2157118X,4,1,P3 Peer Reviewed
Received 29 September 2014
Received in revised form 21 October 2014
Accepted 27 October 2014
Available online 10 December 2014

Amido Black 10B in the Forensic Investigation - Comparative Studies of Forensic Stain Material

P J Laberke [a], *S Ilg* [a], *H-P Bieri* [b], *R Hausmann* [a], *B Balitzki* [*, a]

a Institute of Legal Medicine, University of Basel, Switzerland.
b Forensic Technical Department of the Police Basel-Stadt, Basel, Switzerland.

* Corresponding Author: Dr. P. J. Laberke. Institute of Forensic Medicine, University of Basel, Pestalozzistr. 22, 4056 Basel, Switzerland. E-mail: patrick.laberke@bs.ch

Abstract Bloodstains are among the most meaningful traces in forensic case analysis, which can offer extensive information in forensic case analysis. On the one hand evaluation of the structure, amount, shape and distribution at a crime scene may provide evidence for a reconstruction of events, on the other hand it is now possible to generate a complete DNA profile out of smallest amounts of blood, which can also be used to reconstruct an event and allow conclusions on participation of persons in a crime by molecular genetic analysis. In this study whole blood samples were applied on different substrates and then analyzed for blood using Hexagon OBTI®. Incubation with two different mixtures of Amido Black 10B showed strong adverse effects and false negative results with the OBTI. In contrast no influence of Amido Black 10B was seen in the subsequent DNA analysis.

Keywords: Forensic science, Blood, Indicators and reagents, Sensitivity and specificity, Reliability of results, DNA.

In recent years, a new method has been established in forensics for the visualization and contrast of blood traces, based on the reaction of the blood with Amido Black (Amidoschwarz, AS) 10B. The immunological test principle of Hexagon OBTI® based on the detection of a plasma protein that is also stained by AS 10B. The influence of this staining of blood proteins on the result of the immunological detection of blood and subsequent molecular genetic DNA typing has not been considered.

1 Basics

Traces of blood can be saved in diverse designs at crime scenes [9, 10]. From large pools of blood to micro traces of blood they find themselves in response to the offense at crime scenes in their environment or accomplices to track sources. Now there is a plethora of methods which are available [1, 2, 3, 4, 5, 6, 7, 11, 13, 14, 15] for the visualization and contrast, as well as for the qualitative detection of traces of blood. They are usually used directly at the crime scene and especially for the smallest amounts of blood or visually bad undiscovered tracks, especially in cases where the scene of conspicuous traces of blood already be cleaned .

It can be minimized the amount of blood visible. For daktylo-scopic investigations, the gain in visual perception of tracks may be necessary to document them photographically can. Many methods use the Pseudoper-oxidase reaction of hemoglobin and its derivatives, [2, 11, 15]. In part, this chemiluminescence production increases the visual perception of the trail of blood [14]. The visualization of blood traces by excitation with different light sources is used at crime scenes [13].

After visualizing a trail of blood and its fingerprint analysis, it follows by the actual blood detection using commercial, immunological detection methods, which are characterized at high specificity and sensitivity as well as quick and easy to use[1,3]. The positive detection of blood leads mostly for molecular genetic analysis of the track in order to make a personal assignment.

I has established the individual elements of the blood trail analysis of the visualization, documentation, the blood evidence to DNA typing in forensic trace analysis [2, 11]. Less known are the influences of the methods used in each subsequent analysis. For luminol reagent often-used for the visualization of blood, impairment of subsequent blood detection with the Hexagon OBTI® could be detected [4] as a function of blood volume and application time.

2 Methods

Amido black 10B

Amido black 10B, also known as naphthol blue black B, is an azo dye, which was used as one of the first dyes in gel electrophoresis for visualization of proteins. It has been increasingly used [1] in the fingerprint traces of dye since the beginning of the 90s.

"BDA" -recipe the AS-10B dyeing and rinsing solution

0.4 g AS 10B are dissolved in 180 ml of methanol and 20 ml of glacial acetic acid staining solution for the "BKA" -Recipe. After spraying the dye solution on the track first supports the excess ink with a methanol-glacial acetic acid solution (9: 1), followed by a solution of distilled water and glacial acetic acid (19: 1) and finally rinsed with distilled water.

"The FBI" -recipe the AS 10B dyeing and rinsing solution

For the staining solution according to the "FBI" -Recipe 8.0 g sulfosalicylic acid, 1.2 g and 1.2 g of sodium carbonate AS 10B are successively dissolved in 200 ml of distilled water and then with 20 ml of formic acid, 20 ml of glacial acetic acid and 5 ml Kodak Photo Flo 600® added. The staining solution is sprayed onto the track carrier and then rinsed off the excess paint with distilled water.

Immunological detection of blood

Principle of Hexagon OBTI®

The Occult Blood Test Immunological (OBTI) is a clinical rapid test for the detection of occult blood in stool samples using an immunochromatographic test procedure. According to the manufacturer is the detection limit, based on the transport medium, at 0.1 µg / ml hemoglobin (Hb) and the maximum Hb concentration at 2 mg / ml. The test results can be read after 2-3 min. In the case of a negative result must be a second reading after 10 minutes.

Determination of the optimal blood concentration

To exclude effects of blood concentration of the test result, 0.1, 1, 10 and 100 µl of the whole blood samples were pipetted directly and shaken in 2000 µl OBTI-test-buffer. After 5 s 2 TrpF., placed in the sample window of OBTI Test Cassette. The time from dropping into the sample window to safe reading of a positive test result was documented.

Blood Testing in the presence of amido black 10B

Each 1 µl of the male blood was pipetted on 30 white cotton swabs. Each of these 10 samples were treated with the "BKA" -Recipe and the "FBI" -Recipe; the remaining 10 lobules were left untreated. Samples from each batch were then placed in the OBTI buffer. After 5 s 2 TrpF., added dropwise to the mixture in the sample window until the appearance of the positive result.

In a second step, each 1 ul of the whole blood was pipetted into a total of 30 reaction vessels made of plastic. For each of these 10 samples, the components of the AS 10B staining methods "BKA" and "FBI" were successively added and removed. The remaining 10 samples in the reaction vessels were left untreated. After addition of the OBTI-Buffers in the reaction vessels and 5-second incubation, 2 TrpF. were added to the test cassette.

DNA testing

Extraction

Analogous to the sample preparation for the proof of blood in the presence of AS 10B 60 samples were created and performed DNA extraction with the Investigator® kit (Qiagen). To the manufacturer's protocol was used for isolation of DNA from bodily fluids. The analysis period was 1 h.

Quantification

The PLEXOR®-technology is a "real-time polymerase chain reaction" - (Real-time PCR "-) test procedureallows [8] the quantification of total male DNA at the same time. The DNA-content of the samples was measured with the RotorGene®-system. For this purpose, each 2 µl of each sample were indicated used with the PLEXOR®-HY-system according to the manufacturer's protocol in duplicate and the mean. It was also performed a melting curve determination for the products of autosomal, gonosomal and internal PCR-Control (IPC). The analysis of the raw data was performed using the PLEXOR® HY Analysis Software and forensic analysis settings.

DNA profile

For each series of tests of different pretreatments and carriers of a median of DNA-Concentration sample was exemplary, from a DNA-Profile in 11 STR systems and the gender Amelogeninsystem was created.

3 Results

Determination of the optimal blood concentration

In determining the optimal blood concentration for the OBTI using the investigation of a serial dilution of whole blood from a healthy male subjects the control line appeared immediately in all activities undertaken OBTI trials after contact with the test medium and thus indicated a regular course of the test.

After 30 min, the lowest dilution of 1 : 20 could also be detected. The test was therefore seen as negative. At dilutions of 1: 200 and 1: 20.000 after 13 resp. 18 s only a weak test line was visible; this resulted in the assignment to "weakly positive" result. At a dilution of 1: 2000, a significantly positive test result was already evident after 12 s.

Blood Testing using the OBTI

The comparison of the results in OBTI the untreated and the AS 10B ("BKA" or "FBI") stained blood samples shows in Fig. 1. The results were futher classeified based on their underlying Träger material cotton) Trägermaterialien Baumwolle, BW) or Reaction-vessel (Reaktionsgefäß, RG). Because of the support materials used cotton. A total of 60 blood samples were investigated. For the 20 untreated blood samples for BW and RG, clearly positive test results could be read within 10-22.

After treatment with AS 10B "BKA", it could only have noted 9 BW weak positive and a negative test results; this also had to be considered as negative due to exceeding the maximum test time of 5 weakly positive samples.

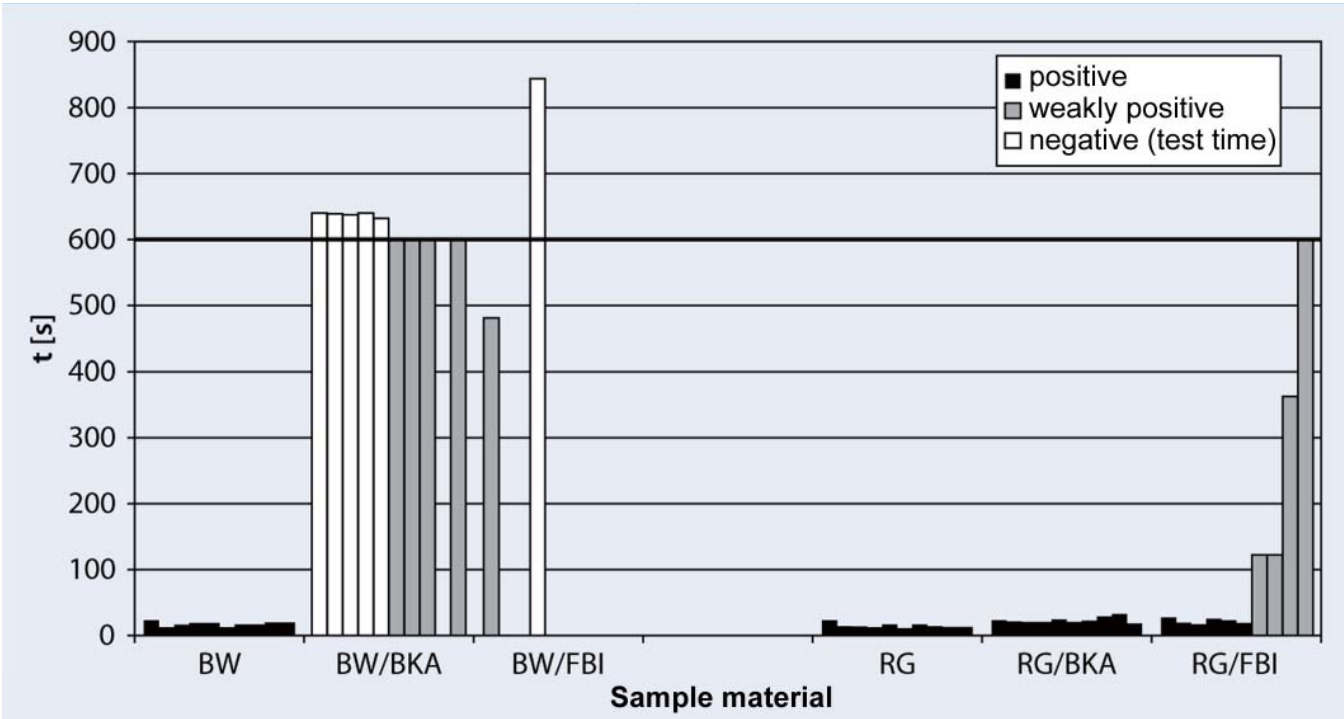

Fig 1. Blood Testing with the OBTI. BKA "BKA" -Recipe for amido black 10B, BW cotton, FBI "FBI" -Recipe for Amido 10B, RG reaction vessel.

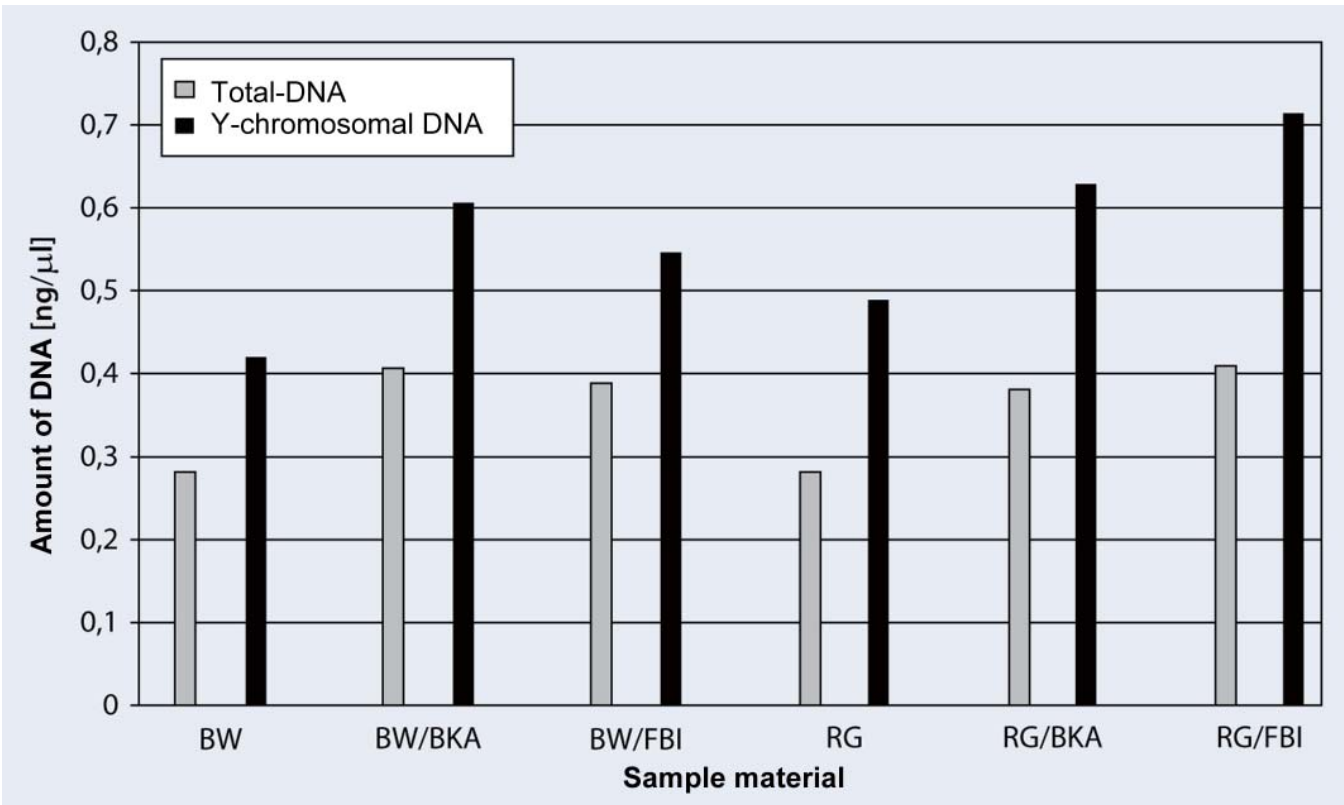

Fig 1. Blood Testing with the OBTI. BKA "BKA" -Recipe for amido black 10B, BW cotton, FBI "FBI" -Recipe for Amido 10B, RG reaction vessel.

These samples are in Fig. 1 shows in white. The 10 samples in the RG gave, after treatment with AS 10B "BKA" with an average response time of 23 s consistently positive results.

Of the 10 blood samples for BW, who had been following the "FBI" -Recipe with AS 10B be-, it is showed only a sample after 8 min of a positive result in OBTI. The weak test line to another sample could be read after 14 min. Because of this reaction time in the test, the negative results has been assigned as all other samples of this series of tests. The blood samples RG from this series of tests were positive with 6 and 4 weakly positive results as well as response times between 18 s and up to 10 minutes of a rather heterogeneous overall result.

DNA quantification

Extraction of DNA was accomplished for all 60 blood samples studied. In addition to the amount autosomal DNA (total DNA) and the amount of Y-chromosomal DNA for each sample and a value for the IPC were determined. The median of the measured amounts of DNA provides (ng / ul extracted DNA) and its dependence on the applied staining method and the different carriers as Fig. 2 illustrated.

Melting curve provisions and total DNA

All completed melting curve provisions confirmed the specificity of the amplification. In quantification of total DNA from whole blood was applied to BW in the native sample from 0.11 to 0.63 ng / µl DNA (median 0.28 ng / µl) were identified. For the "BKA" samples coloring on BW, it was measuable between 0.32 and 0.71 ng / µl of total DNA (median 0.41 ng / µl), and for using the "FBI" samples, between 0.18 and 0.0.66 ng / µl of total DNA (median 0.39 ng / µl).

The untreated samples reported in RG between 0.17 and 0.64 ng / µl of total DNA (median 0.28 ng / µl). After staining using the "BKA" - and "FBI"- method, here could a median of 0.38 ng / µl of a total DNA (variation from 0.10 to 1.12 ng / µl), respectively, a total DNA of 0.41 ng / µl in (variation from 0.15 to 0.88 ng / µl) are detected.

Y-chromosomal DNA

To the native BW sample, the evidence left between 0.17 and 1.41 ng / µl Y-chromosomal DNA (Median 0.42 ng / µl). The sample treated with AS 10B "BKA" showed 0.39 to 0.95 ng / µl (median 0.61 ng / µl), while the sample with the "FBI" mixture from 0.17 to 0.83 ng / µl (median 0.55 ng / µl) Y-chromosomal DNA.

The native sample in the RG had a content of Y-chromosomal DNA from 0.31 to 0.98 ng / µl (median 0.49 ng / µl). With the addition of AS 10B "BKA", values were 0.26 to 1.31 ng / µl (median 0.63 ng / µl), with the addition of AS 10B "FBI" 0.32 to 1.20 ng / µl (median 0.72).

DNA profiles

For all selected samples a complete male profile could not be created.

4 Discussion

Determination of the optimal blood concentration

False-negative or weakly positive results of OBTI are already described in the literature in both large and too low blood levels [3, 4]. This observation was confirmed when determining the optimal blood concentration for the implementation of further experiments. At higher concentrations of human hemoglobin (hHb) in the sample of immobilized anti-hHb antibody unbound hHb can be occupied, making a false-negative result is due (Hook effect). This effect was at 1: 20 dilution observed. A negative test result must therefore be confirmed with a dilution of the sample. The dilution of 1: 200 initially showed no effect on the reaction time (13 s). The color intensity of the forming test line was, however, significantly affected, so the result was "weak positive" classified in the category. The result for the 1: 20.000 dilution also showed the effect of the decreasing intensity of the test line and was therefore assigned to the category "weakly positive".

The optimal dilution proved to be a mixture ratio of 1: 2000, in which, after a few seconds a positive result could be read. This value varies depending on the study - partly were still at dilutions of 1 : 100.000 described clearly positive results [3, 4]. One possible explanation for this could be the lack of information provided by the manufacturer for optimum working temperature of OBTI. The storage of the kit components is recommended at 2-25°C. Whether the test after previous cooling should only obtain room temperature, is not specified. An influence of the temperature of the kit when applied to the sensitivity is conceivable in principle in immunological methods.

Blood Testing using the OBTI

The results for the untreated blood sample in the ratio 1: 2000 revealed no influence of the substrate on the functionality of the OBTI. For all samples within a few seconds clear positive test result was read.

During a meeting of experts "crime scene" in 2007, different formulations of the dye in securing traces of blood were tested and evaluated in AS 10B as a result of especially suitable dye for various track carriers [6]. The experts of the forensic favor an investigation to make the "BKA" -Recipe providing for the solution of the dye in methanol and glacial acetic acid. As an alternative method, especially for working without methanol, the "FBI" -Recipe is given [6]. In addition, there is a wealth of other provisions to prepare the working solution and rinse solutions to be used, the excess,

unbound to proteins dye should be removed from the track carriers. In the application of the dye solution, there are a number of techniques - from the spraying of the track support to the use of immersion baths [1, 6].

The staining with AS 10B "BKA" - and "FBI" -recipe and the use of various trace carriers have partly clear influence on the OBTI blood evidence. Using the track girder RG and consistently positive results for the "BKA" coloring no influence on the sensitivity of the OBTI could be derived. With the "FBI" mixture at least 60% of the samples analyzed in the RG rendered still significantly positive test result.

In contrast, it has been demonstrated that the use of AS-10B recipes with traces of blood on BW predominantly leads to false-negative or only weakly single-positive results, thus limiting the value of OBTI severely limited in terms of pre-tests or no longer exists.

Which chemicals used in the different staining techniques responsible for this result could not be verified on the basis of this study. The effect of different track support is readily explicable that the solutions used can be absorbed by the cotton and washed out with the bad rinsing solutions than be possible in the reaction vessel.

DNA analysis

With regard to the determination of the amount of total and Y-chromosomal DNA in the studies wide variations were between samples of the sub-groups, which, however, showed (Fig. 2) in the overall view of a similar median. These fluctuations can be explained informally by the different cell number of the applied whole blood samples in which no previous cell count was performed. Striking was the over content of total DNA median amount of Y-chromosomal DNA at all quantifications. This could be interpreted as the variance of the PCR. The values are within the natural biological variation between 0.4 and 2.0 for the ratio of autosomal for Y chromosomal DNA of male subjects.

Ultimately, however, in each case, "short tandem repeat" - (STR) analysis was carried out in which no influence could be observed in other studies [12] and other chemicals to the blood evidence seen for AS 10B.

5 Conclusions

For the practical use of AS 10B in the forensic analysis, i is important to note that, in previous application on alleged bloodstains, strong limitations or false-negative results of OBTI may occur. The use of test methods in which the Pseudoperoxidas Reaktion of Hb results in a color change of an initially colorless substance (benzidine, luminol, Leukomalachit etc.) by transfer of liberated oxygen, could offer an alternative. On the possibility to create a DNA profile that blends used "BKA" and "FBI", however, it seems to have no influence. In this respect, the DNA analysis still runs samples in cases of a negative OBTI to relevant results and should always be carried out in case of doubt.

References

[1] Blumenberg C. Daktyloskopische Spurensuche – Amido-Schwarz für blutige Fingerspuren. Kriminalistik, 1991, 8-9: 547-566.
[2] Gaensslen RE. Identification – catalytic tests. In: Gaensslen RE (Hrsg) Source book in forensic serology, immunology and biochemistry. U.S. Department of Justice, United States Government Printing Office, Washington DC, 1983, S: 101-145.
[3] Hermon D, Shpitzen M, Oz C et al. The use of Hexagon OBTI test for detection of human blood at crime scenes and on items of evidence. J Forensic Identif, 2003, 53: 566-575.
[4] Hochmeister MN, Budowle B, Sparkes R et al . Validation studies of an immunochromatographic 1-step test for the forensic identification of human blood. Forensic Sci, 1999, 5: 597-602.
[5] Klein A, Feudel E, Türk E et al. Lumineszenz nach Luminolanwendung. Rechtsmedizin, 2007, 17: 146-152.
[6] Lehmann P . Sichtbarmachung blutiger Fingerspuren mittels unterschiedlicher Amidoschwarz-Ansätze und Acid-Yellow. Expertentagung Tatortarbeit – Spurensicherung LKA, Baden-Württemberg, 2007.
[7] Nielsen K, Mogensen HS, Hedman J et al. Comparison of five DNA quantification methods. Forensic Sci Int, 2008, 2: 226-230.
[8] Krenke BE, Nassif N, Sprecher CJ et al. Developmental validation of a real-time PCR assay for the simultaneous quantification of total human and male DNA. Forensic Sci Int, 2008, 3: 14-21.
[9] Peschel O, Mützel E, Rothschild MA (2008) Blutspurenmuster-Verteilungsanalyse. Rechtsmedizin 18: 131-146.
[10] Peschel O, Rothschild MA, Mützel E. Blutspuren bei Schussverletzungen. Rechtsmedizin, 2010, 20: 91-97.
[11] Ponce AC, Pascual FAV. Critical revision of presumptive tests for bloodstains. Forensic Sci Commun, 1999, 1: 1-5.
[12] Poon H, Elliott J, Modler J, Frégeau C. The use of Hemastix® and the subsequent lack of DNA recovery using the Promega DNA IQ TM System. J Forensic Sci, 2009, 54: 1278-1286.
[13] Seidl S, Hausmann R, Betz P. Comparison of laser and mercury-arc lamp for the detection of body fluids on different substrates. Int J Legal Med, 2008, 122: 241-244.
[14] Thorogate R, Moreira J, Jickells S et al. A novel fluorescence-based method in forensic science for the detection of blood in situ. Forensic Sci Int, 2008, 2: 363-371.
[15] Tobe SS, Watson N, Daeid NN. Evaluation of six presumptive tests for blood, their specificity, sensitivity, and effect on high molecular-weight DNA. J Forensic Sci, 2007, 52: 102-109. ■