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DNA-PCR Analysis of Bloodstains Sampled by the Polyvinyl-Alcohol Method

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ABSTRACT: Among the usual techniques of sampling gunshot residues (GSR), the polyvinyl-alcohol method (PVAL) includes the advantage of embedding all particles, foreign bodies and stains on the surface of the shooter's hand in exact and reproducible topographic localization. The aim of the present study on ten persons killed by firearms was to check the possibility of DNA-PCR typing of blood traces embedded in the PVAL gloves in a second step following GSR analysis. The results of these examinations verify that the PVAL technique does not include factors that inhibit successful PCR typing. Thus the PVAL method can be recommended as a combination technique to secure and preserve inorganic and biological traces at the same time.

KEYWORDS: forensic science, PVAL, polyvinyl alcohol, gunshot residues, DNR-PCR typing, individualization

During the examination of firearm victims, usually the question has to be answered whether the shots could have been self-inflicted, either intentionally or accidentally. An essential procedure is to search for gunshot residues or blood traces on the victim's hands.

Sampling gunshot residues (GSR) from the hands (1–3) is performed using various materials: cotton-swabs, tape-lifts, adhesive foils and polyvinyl alcohol. GSR sampling can be problematic in the presence of blood. Cotton swabs moistened with bidistilled water do not retain enough GSR if bloodstains are wet or liquid. Also, the mechanical resistance of the swabs does not allow scrubbing off dried blood. Tape-lifts, which are optimized for the examination by scanning electron microscopy, and films have an adhesive surface. The adhesive capacity is quickly exhausted by wet blood traces. Therefore the content of GSR in these samples is reduced in comparison with clean hands (4).

The PVAL method introduced by Merkel and Mailaender (5) uses polyvinyl alcohol (PVAL), a liquid polymer, which models the hand and embeds all particles, foreign bodies and stains on the surface of the hands (Figs. 1 and 2). After drying, the PVAL layers with the embedded traces can be completely removed from the hand like a glove. This results in a topographically true conservation of both GSR and bloodstains embedded in the PVAL ''glove'' (Fig. 3).

The method is suitable for the detection and characterization of GSR (4,5), but has not been tested for its capability of an additional DNA-PCR typing of bloodstains embedded in the same PVAL glove. The intent of this study was to examine if the PVAL method includes factors which inhibit PCR profiling.

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Material

The study had exclusively scientific objectives without any evidential contribution. The police allowed the experiments on condition that the autoptic procedure was not disturbed. Thus it was decided to prepare the PVAL hand casts after the autopsies. For the securing of trace material, both hands were covered by big envelopes during transport and autopsy. Since plastic bags can create detrimental humidity, envelopes made of thick paper were preferred.

Ten cases of death by firearms were included in the study where the case situations allowed a clear classification as suicide, homicide or accident. In eight cases the shot wounds were self-inflicted, either intentionally (five cases) or accidentally (three cases). From the circumstances it could be expected in these cases that the hands of the victims were contaminated with both GSR and blood. Two cases of homicide (cases #4 and #9) served as the negative control. In these cases a contamination with GSR or blood was unlikely, according to the scene of crime (Table 1). The firearms confiscated by the police were mostly handguns. Only one man used a small-bore rifle (.22 lr) for suicide. Tables 2 and 3 show the calibers and ammunition involved.

In nine cases during autopsy a blood sample from the femoral vein and, in one case, muscle tissue were taken as reference materials. The samples were frozen until DNA examination.

Methods

Polyvinyl alcohol (Sigma P 1763) was prepared as 10% solution. Liquid medium was 20% ethanol in which polyvinyl alcohol was stirred at 70 to 80°C. At room temperature the PVAL solution was applied with a fine new paintbrush on each hand in three layers that were dried by a hair dryer. The first layer was gently dotted, beginning at the little finger and going to the thumb. The second layer was painted as well. In the third layer, a stabilizing layer of cotton gaze was embedded. After 20 min of terminal drying, the PVAL coating was removed so that the inner side was reversed to exterior. The PVAL hand cast needed another 60 min of self-drying and eventually got the form of a glove.

Microradiography (Faxitron M55 [Hewlett Packard]) was the first step of examination. High-resolution radiographs revealed the fine metallic particles (10 to 100 μm). With regard to the long time of exposure (at least 2 min), the PVAL gloves had to be completely dried to avoid motion artifacts.

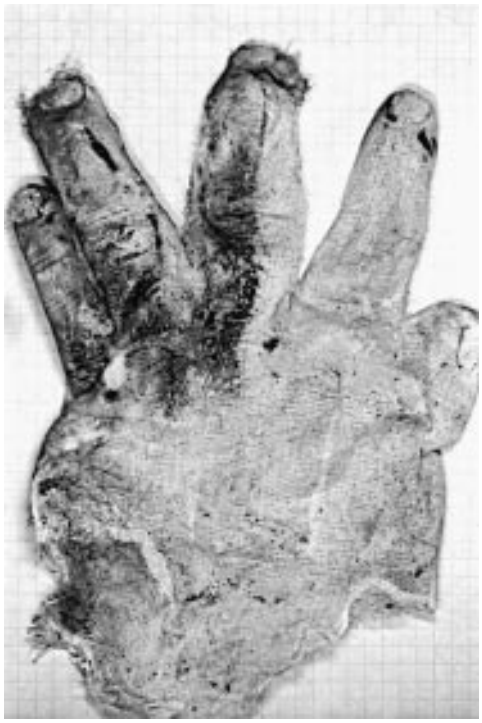

FIG. 1—*PVAL glove (case #5), dorsum of right hand.*

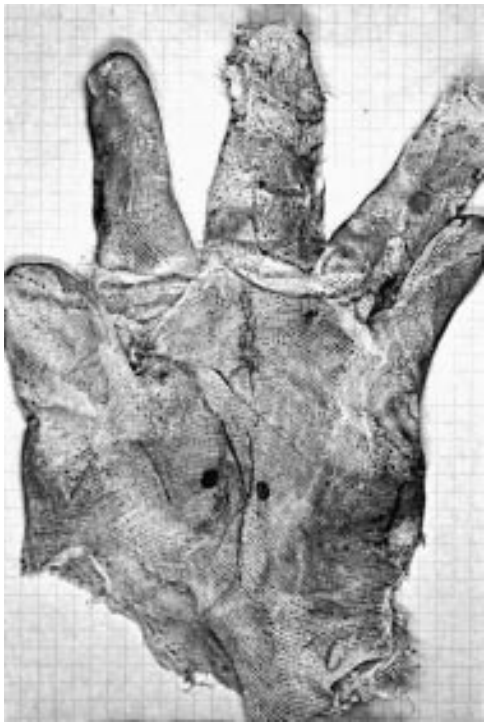

FIG. 2—*PVAL glove (case #5), palm of same hand.*

For chemographic detection of GSR it was necessary to know the primer composition of the cartridge used. This was tested by energy dispersive X-ray fluorescence analysis using MESA 500 (Horiba). In cases with lead-containing primers, the glove was sprayed with 2% tartaric acid and then with freshly prepared saturated sodium rhodizonate solution. The cartridges with lead-free primers (Sintox®) had been produced by RWS (Dynamit Nobel, Troisdorf, Germany) and contained titanium and zinc. In these cases, alkaline zincon was applied by spraying as indicated by Beijer (6).

If macroscopical examinations of PVAL gloves resulted in suspected contamination with blood, parts with a size of approximately 9 mL^2 were cut from the suspicious area. DNA isolation was started by soaking in 200 μL PBS buffer (0.15 molar, pH 7.5). For the following extraction steps the QiAmp DNA isolation kit (Qiagen) was used according to the instructions of the manufacturer (7). Quality and quantity of the isolated DNA were checked on 0.6% agarose gels containing ethidium bromide. Depending on the content of DNA, 2 to 8 μL DNA solution was used for PCR (approximately 4 to 5 ng DNA per sample).

No pretests for the presence of blood were performed to avoid disturbances. Only obviously contaminated areas were cut out.

FIG. 3—*PVAL glove, blood contamination of dermal ridge pattern.*

TABLE 1—*Circumstances of death by firearm.*

Circumstance of Death	Homicide	Accident	Suicide	All
Number of cases	2	3	5	10
Males	0	2	3	5
Females	2	1	2	5

TABLE 2—*Caliber and firearms.*

Caliber	.22 lr	.25	.32	.38	9 mm × 19*
Number of cases	2	1	3	1	3
Revolver			1	1	
Pistol	1	1	2		3
Rifle	1				

* 9 mm × 19 is the pistol caliber 9 mm Luger.

TABLE 3—*Ammunition.*

Bullet	Lead	Semi Metal Jacket	Full Metal Jacket
Number of cases	2	2	6

Since unsuspicious areas were not necessarily free of traces of blood, they could not serve as negative control. In the homicide cases without obvious contamination, randomly selected areas were cut out. Even if the sample appeared negative on the agarose gel in the homicide cases, amplification was tried.

The isolated DNA was amplified for HumTH01 (FK01 Kit, SERAC)), HumVWA31 (FK02 Kit, SERAC, Germany), HumFES (FK03 Kit, SERAC, Germany), HumF13B (FK04 Kit, SERAC, Germany), HumCD4 (FK05 Kit, SERAC, Germany), HumFGA (FK06 Kit, SERAC, Germany), D1S80 (AmplFLP TM D1S80 PCR Amplification Kit, Perkin Elmer, USA), HLA-DQα, LDLR, GYPA, D7S8, HBGG and GC, (Perkin-Elmer AmpliType™ PM + DQA1 PCR amplification and typing kit, Perkin Elmer, USA). Amplification conditions, electrophoresis and silver staining (STR and VNTR systems) have been previously described in the manufactures' protocols (8–15), except that 16 μg bovine serum albumin was added to the PCR. Amplification was carried out on a Perkin Elmer DNA Thermal Cycler 480.

Statistical Analysis

The probability of identity—based on an a-priori of 0.5 (16,17)—and the frequency rank of the typed DNA genotypes found in the blood samples (muscle tissue) and specimens from PVAL gloves were estimated by use of the software PCR Biostatistik 1.1 (18). Gene frequencies were taken from pooled German samples, calculated by weighted arithmetical means (19).

TABLE 4—*Patterns of bloodstains in PVAL gloves (multiple countings are possible); number of observations.*

Pattern	Right Hand	Left Hand
Diffused moisture	8	7
Macro spatter	3	
Micro spatter	2	2
Trickle	2	2
Not visible	2	2

Results

As expected, in all eight cases where the shot wounds were self-inflicted—either intentionally or accidentally—GSR could be detected on the PVAL gloves, whereas in the two cases of homicide the tests were negative.

In the eight cases with self-inflicted shot wounds also blood-like contaminations could be found macroscopically, either as diffused moisture, macro spatter, micro spatter, or trickle, respectively (Table 4). From all of the 15 PVAL gloves belonging to these cases DNA could be isolated. DNA was typed for 13 PCR systems: one VNTR system, six STR systems, and six structure polymorphisms. The results are listed in Table 5. The frequency ranks of the genotype combinations found in samples from PVAL gloves and the reference blood (tissue) samples of the victims are listed in Table 6. In these eight cases all results received from the PVAL gloves corresponded to the findings in blood or tissue samples of the respective victims. The degrees of identity probability are shown in Table 6. In all cases more than 99.999% could be calculated.

In the two homicide cases no DNA could be extracted and no amplification products were found.

Discussion

PVAL gloves represent true images of the original findings on the hands. This means that the location of GSR on the hands can precisely be reconstructed at any time. This is a decisive advantage over other techniques of GSR sampling. The PVAL method is

TABLE 5—*DNA-STR typing on blood/muscular tissue of corpses and assigned PVAL gloves (☞); ∅ typing not successful; n.t. not tested.*

Case	Material	HLA DQα	LDLR	GYPA	HBGG	D7S8	GC	D1S80	VWA	FES	F13B	THO1	Fibra	CD4
1	Blood	3,3	AB	AB	3,1	AA	AA	22,24	15,16	10,11	8,9	6,9	n.t	6,6
	∅ left	∅	∅	∅	∅	∅	∅	22,24	15,16	10,11	∅	6,9	n.t	6,6
	☞ right	3,3	∅	AB	BB	AA	AA	22,24	15,16	10,11	∅	6,9	n.t	6,6
2	Blood	1,1	BB	AB	AA	AB	AC	20,21	16,16	12,12	6,10	9,9.3	21,21	6,6.1
	☞ left	1,1	BB	AB	AA	AB	AC	∅	16,16	12,12	6,10	9,9.3	21,21	6,6.1
	☞ right	1,1	BB	AB	AA	AB	AC	∅	16,16	12,12	6,10	9,9.3	21,21	6,6.1
3	Blood	4,4	AB	AA	BB	AB	CC	18,24	17,19	11,11	10,10	8,9.3	22,24	6,10
	☞ right	4,4	AB	AA	BB	AB	CC	18,24	17,19	11,11	10,10	8,9.3	22,24	6,10
4	Blood	1.1-1.3	AB	AB	AB	AB	AA	18,18	18,18	11,11	8,8	9,9	22,24	6,6
	☞ left	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅
	☞ right	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅
5	Blood	1.1-4	AB	AA	AB	AB	CC	24-31	16-17	11-12	8,8	8,9.3	n.t	n.t
	☞ left	1.1-3	AB	AA	AB	AB	CC	24-31	16-17	11-12	8,8	8,9.3	n.t	n.t
	☞ right	1.1-4	AB	AA	AB	AB	CC	24-31	16-17	11-12	8,8	8,9.3	n.t	n.t
6	Blood	1.2.2	AB	AB	AB	AB	n.t	18,28	14,17	10,13	n.t	6,9	n.t	6,10
	☞ left	1.2.2	AB	AB	AB	AB	∅	18,28	14,17	10,13	n.t	6,9	n.t	6,10
	☞ right	1.2.2	AB	AB	AB	AB	∅	18,24	∅	10,13	n.t	6,9	n.t	6,10
7	Blood	2,4	BB	BB	BB	BB	BB	27,31	17,17	10,13	8,9	6,6	n.t	n.t
	☞ left	2,4	BB	BB	BB	BB	BB	27,31	17,17	10,13	8,9	6,6	n.t	n.t
	☞ right	2,4	BB	BB	BB	BB	BB	27,31	17,17	10,13	8,9	6,6	n.t	n.t
8	muscle	∅	BB	AB	BB	AB	∅	n.t	14,16	11,11	8,10	9,9.3	19,20	n.t
	☞ left	∅	BB	AB	BB	AB	∅	n.t	16,16	11,11	10,10	9,9.3	19,20	n.t
	☞ right	∅	BB	AB	BB	AB	∅	n.t	14,16	11,11	10,10	9,9.3	19,20	n.t
9	Blood	n.t	n.t	n.t	n.t	n.t	n.t	n.t	n.t	n.t	n.t	n.t	n.t	n.t
	☞ left	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅
	☞ right	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅
10	Blood	1.1-1.2	AB	BB	BB	AB	CC	24-34	16-19	10,11	8,9	6,9	21,22	6,6
	☞ left	1.1-1.2	AB	BB	BB	AB	CC	24-34	16-19	10,11	8,9	6,9	21,22	6,6
	☞ right	1.1-1.2	AB	BB	BB	AB	CC	24-34	16-19	10,11	8,9	6,9	21,22	6,6

TABLE 6—*Frequencies of the detected DNA characteristics and probabilities of identity (case numbers 4 and 9 omitted; homicides, no DNA found).*

Case No.	material	frequency rank	probability of identity (%)
1	blood	1: [illegible]	
	ex left	1: [illegible]	[illegible]
	ex right	1: [illegible]	[illegible]
2	blood	1: [illegible]	
	ex left	1: [illegible]	[illegible]
	ex right	1: [illegible]	[illegible]
3	blood	1: [illegible]	
	ex left	1: [illegible]	[illegible]
5	blood	1: [illegible]	
	ex left	1: [illegible]	[illegible]
	ex right	1: [illegible]	[illegible]
6	blood	1: [illegible]	
	ex left	1: [illegible]	[illegible]
	ex right	1: [illegible]	[illegible]
7	blood	1: [illegible]	
	ex left	1: [illegible]	[illegible]
	ex right	1: [illegible]	[illegible]
8	blood	1: [illegible]	
	ex left	1: [illegible]	[illegible]
	ex right	1: [illegible]	[illegible]
10	blood	1: [illegible]	
	ex left	1: [illegible]	[illegible]
	ex right	1: [illegible]	[illegible]

more expensive than conventional methods. However, because of this advantage, the technique should be preferred and will be accepted.

But there is another advantage of the PVAL method. Besides GSR, biological traces also are transferred from the hands to the PVAL gloves. The use of other sampling techniques deteriorates the feasibility of additional biological examinations. During the conventional securing of evidence it has to be decided which kind of investigation is of more importance for the reconstruction of the events: securing of material for GSR or for DNA typing. Commonly the bloodstains are exterminated.

The PVAL technique opens new horizons. Both GSR and bloodstains transferred to the PVAL glove are preserved, not only macroscopically by substance but also by precise localization. Moreover, it could be demonstrated that neither the production of PVAL hand cast gloves nor the GSR screening procedure influences the quality of the extracted DNA and the polymerase chain reaction, which is the commonly used technique on the sector of microstains (18).

In the eight cases (15 gloves) with self-inflicted shot wounds, DNA could be isolated from the gloves after GSR screening. DNA-PCR typing was successful in more than 50% of the DNA loci investigated. In all cases, complete identity was found between bloodstains on the PVAL gloves and blood (muscle tissue) samples taken from the assigned corpses. Biostatistical evaluation of the genotype combinations typed in each case on PVAL gloves and blood resulted in nearly all cases in frequencies less than 1 in 1 000 000 persons. The resulting probabilities of identity were estimated at more than 99.999%.

In the two homicide cases (four PVAL gloves), GSR screening was negative and no DNA could be isolated. This finding is of special interest. During the production of the PVAL gloves no hand protection was used by the investigator, in order to avoid contamination by foreign substances such as talcum. The negative DNA results obviously show that no artificial DNA contamination from the investigator's hands occurred. Also, no DNA-containing cutis cells from the corpse were transferred.

The results of this study demonstrate that the PVAL technique enables the simultaneous securing of GSR and bloodstains. For GSR screening as well as for DNA typing, the topography of the original trace carrier is conserved. DNA quality is not influenced by the procedure. For these reasons, the PVAL method can be recommended as a combination technique to secure and preserve inorganic and biological traces.

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