

The ‘triple contrast’ method in experimental wound ballistics and backspatter analysis

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Abstract In practical forensic casework, backspatter recovered from shooters’ hands can be an indicator of self-inflicted gunshot wounds to the head. In such cases, backspatter retrieved from inside the barrel indicates that the weapon found at the death scene was involved in causing the injury to the head. However, systematic research on the aspects conditioning presence, amount and specific patterns of backspatter is lacking so far. Herein, a new concept of backspatter investigation is presented, comprising staining technique, weapon and target medium: the ‘triple contrast method’ was developed, tested and is introduced for experimental backspatter analysis. First, mixtures of various proportions of acrylic paint for optical detection, barium sulphate for radiocontrast imaging in computed tomography and fresh human blood for PCR-based DNA profiling were generated (triple mixture) and tested for DNA quantification and short tandem repeat (STR) typing success. All tested mixtures yielded sufficient DNA that produced full STR profiles suitable for forensic identification. Then, for backspatter analysis, sealed foil bags containing the triple mixture were attached to plastic bottles filled with 10 % ballistic gelatine and covered by a 2–3-mm layer of silicone. To simulate backspatter, close contact shots were fired at these models. Endoscopy of the barrel inside revealed coloured backspatter containing typable DNA and radiographic imaging showed a contrasted bullet path in the gelatine. Cross sections of the gelatine core exhibited cracks and

fissures stained by the acrylic paint facilitating wound ballistic analysis.

Keywords Gunshot wounds · Wound ballistics · Suicide · Firearms · Forensic medicine · Forensic genetics

Introduction

Examinations in cases of death following gunshot injury are a common task for forensic experts. The predominant objective in such cases is to clarify whether the gunshot wound is self-inflicted. Gunshot residues (GSR) collected from the hands of the deceased can indicate that a gun had been fired by that person. Especially, topographical collection and analysis of GSR is a valuable method to produce suitable evidence [1–3].

However, the hands of the deceased represent an area of interdisciplinary forensic interest. Commenting on gunshot effects with regard to ballistics, forensic medicine and criminalistics, Sellier [4] recommended that shooters’ hands should also be examined for biological traces, already in 1969. This still current advice [1] is based on medico-legal experience that in some instances of gunshot injuries, splashes of blood and/or tissue, so-called backspatter, can be observed on shooters’ hands. Backspatter is considered to consist of droplets of biological material, mostly blood, propelled from the entry wound and back against the direction of fire. In 1931, Weimann demonstrated the evidential value of backspatter in determining the hand that had fired the gun [5].

Of particular interest in the investigation of backspatter is its deposition on the inside surface of gun barrels. In 1934, Brüning and Wiethold first reported on the examination of suicide weapons exhibiting traces of biological material on outside and inside surfaces [6]. These observations were later

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confirmed by other groups [7–9] and recently, the morphological findings established via endoscopy of the barrel insides could be verified by PCR analysis also proving the persistence of DNA in consolidated backspatter [10–12].

However, many authors of studies on gunshot suicide emphasize the heterogeneity of backspatter and its lacking in some cases [13, 14]. In fact, the physical conditions and properties of backspatter generation, distribution and consolidation have not been fully elucidated yet, and a systematic experimental approach is lacking so far. Therefore, the authors propose a new experimental concept: tracing the projectile from gun to target where it decelerates and finally resides and in the process documenting all effects and alterations caused by the shot. This comprehensive concept includes analysis of the ballistic model representing the body (or head) as well as the biological traces recovered from inside and/or outside surfaces of the weapon.

In wound ballistics, ballistic gelatine is a widely recognized simulant for biological tissues [14]. Reliable measurements of energy transfer can be performed using the ‘colour contrast method’: acrylic paint sealed into thin foil bags is drawn into the gelatine block by the suction resulting from the collapsing temporary wound cavity [15, 16], thereby filling and staining the emerging cracks and fissures. Preliminary experiments had shown that the paint was also sputtered onto the gun, indicating the method’s potential to simulate backspatter.

Most typically, suicidal gunshots aim to the head and are fired at close contact. In order to closely mimic this scenario, the overly simplistic gelatine block was replaced by a head model. In contrast to gelatine, these complex models (i.e., hollow spheres filled with ballistic gelatine and covered with layers of silicone) are intransparent so that a nondestructive technique of examination is required for preservative wound ballistic analysis. Previous work demonstrated that adding barium sulphate to acrylic paint facilitates parallel imaging via computed tomography (CT) producing satisfactory radiocontrast [17].

In addition, molecular biological analysis of backspatter employed multiplex short tandem repeat (STR)-PCR to successfully detect microspatter below the macroscopic detection threshold. Multiplex STR-PCR is the standard forensic technique for DNA-based identification. In these previous experiments, ballistic models comprising foil bags filled with human blood were used to simulate backspatter [18, 19].

The aim of this study was to investigate whether the integration of human blood in the mixture of acrylic paint and barium sulphate as used in the established radio-colour contrast method had detrimental effects on DNA integrity and STR typing success rate and, vice versa, whether the presence of blood in the mixture derogates optical or radiocontrast-based visualization.

Material and methods

Blood and mixed samples

Blood samples were taken by venipuncture. No EDTA was added, and blood samples were used immediately after collection. A separate set of blood samples was heparinized. All blood samples used in this study were donated by adult, informed and consenting volunteers and the study design was approved by the ethics committee of the Hospital of the University Bonn. Blood was mixed with acrylic paint (CPM, Erkrath, Germany) and/or barium sulphate-based radiocontrast agent Micropaque® (Guerbet, Brussels, Belgium) and sealed into thin 5×5 cm² foil bags.

Weapons, ballistic model and sampling procedure

In this study, ten individual handguns, all duty weapons, six revolvers (cal. 38 special) and four semi-automatic pistols (cal. 9 mm Luger) were used. Calibre 38 special lead round nose bullets (10.2 g) and 9-mm Luger full metal jacketed bullets (10.2 g) were fired.

0.5 l polyethylene bottles were chosen as simple version of a target model. One thin foil bag containing a mixture of fresh blood, acrylic paint and radiocontrast agent was attached to each bottle which was then covered with a 2–3-mm-thick layer of silicone and stored at 4 °C for 16 h. Finally, 10 % gelatine ‘Ballistic III’ (Gelita, Eberbach, Germany) was prepared following Fackler’s instructions [20], filled into the bottles (Fig. 1) and stored at 4 °C for another 36 h. Shots were fired at the model construction with close contact of the muzzle.

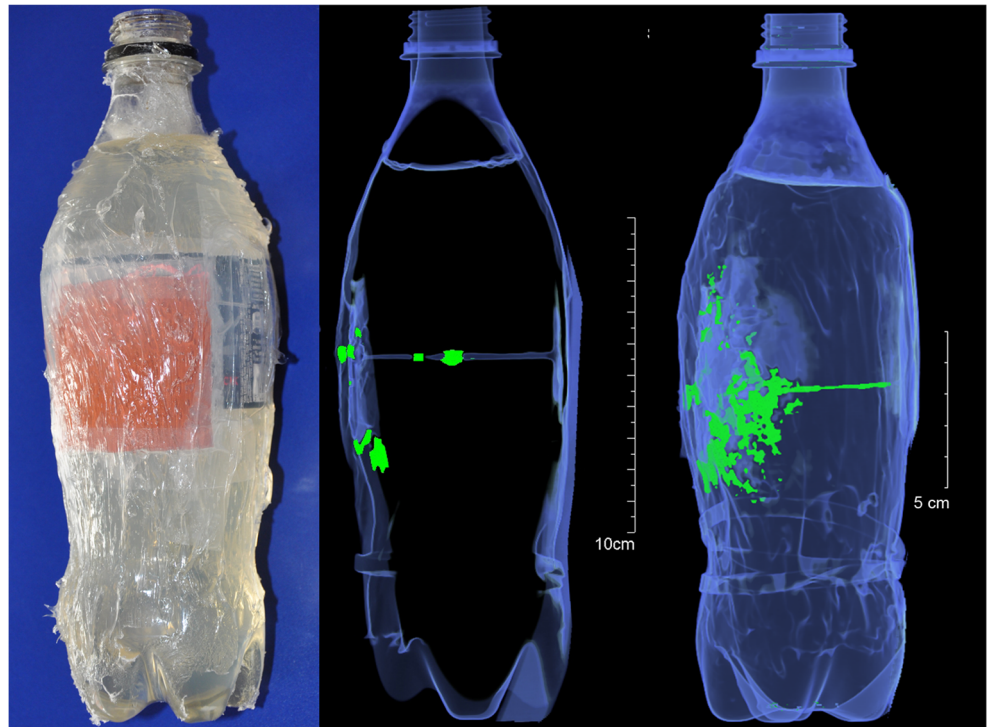
The barrels were examined endoscopically using a 21.5-cm-long ‘Technoscope’ (Karl Storz, Tuttlingen, Germany). Samples were collected using sterile, DNA-free cotton swabs moistened with sterile, desalted water to wipe the inner surface of the barrel of the firearm.

DNA extraction, quantification and detection of PCR inhibitors

DNA was extracted from all backspatter trace samples using the magnetic bead-based PrepFiler Forensic DNA Extraction Kit (Life Technologies, Darmstadt, Germany) according to the manufacturer’s prescriptions.

DNA concentration and the presence of PCR inhibitors was measured by quantitative PCR (qPCR) using the Quantifiler Human DNA Quantification Kit on a 7500 Sequence Detection System (both Life Technologies) as per manufacturer’s recommendation.

Fig. 1 Ballistic model. *Left panel:* A foil bag containing the triple mixture attached to a 0.5-l polyethylene bottle filled with ballistic gelatine and covered with a silicone layer. *Middle panel:* multiplanar reconstruction showing the bullet path from left to right. *Bright spots* correspond to high concentrations of radiocontrast agent. *Right panel:* volume rendering of another bottle. The rest of radiocontrast agent in the shredded foil bag is visible at the front



STR-Multiplex PCR and fragment detection

PowerPlex® ESX 17 and ESI 17 kits (Promega, Madison, WI, USA) were used to conduct multiplex STR-PCR, following the instructions provided by the manufacturer. Fragment separation and detection was performed on a 310 Genetic Analyzer (Life Technologies); data analysis was done using the GeneMapper software v3.2 (Life Technologies).

Radiological imaging of projectile paths in the bottle models

About 24 h after shooting tests, the target models were scanned with a CT Somatom Definition AS 64 (Siemens, Germany) using following settings: 100 kV, 120 mA, slice 0.6 mm, kernels J30s and H70h. The image analysis was performed using multiplanar reconstruction on a Syngo CT workstation (Leonardo).

Means to avoid contamination

All work described herein was conducted wearing gloves and an aerosolproof facemask. The endoscope was cleaned with isopropyl alcohol and DNA-ExitusPlus™ (AppliChem, Darmstadt, Germany) following manufacturer's instructions.

The guns, used in this study, were thoroughly cleaned before being used to deliver any experimental shot. Briefly, a special cleaning rod with attached barrel cleaners of woollen felt (VFG, Giengen, Germany) that had been soaked with purpose-made oil 'WD-40' aerosol (WD-40 Company,

Milton Keynes, UK) was used to rigorously rub and wipe the barrel from the inside and finally treated with DNA-ExitusPlus™. This procedure proved to be sufficient to quantitatively remove any traces of typable DNA from the barrel (data not shown).

Results

DNA analysis from duplex and triplex mixtures

In a preliminary experiment, blood and acrylic paint were mixed in various proportions (duplex mixtures) and DNA yields and STR typing quality was directly assessed from six fresh and six dried samples of these mixtures. PCR inhibition was detectable in five fresh and two dried samples, but all samples yielded quantifiable DNA sufficient for STR typing. STR typing resulted in full profiles in both tested samples containing 50 and 30 % blood, respectively, and regardless of the presence of inhibition. Supplementary Table 1 summarizes the results.

Sensitivity of STR typing from dried samples of 50:50 duplex mixtures was then assessed in a five-step dilution series comprising samples that contained from 50 to 1 µl of blood. STR typing resulted in full profiles even from minute amounts of blood (Supplementary Table 2).

Table 1 presents the results of DNA quantification and STR typing in dried samples of triplex mixtures

Table 1 DNA quantification and STR typing in mixtures of blood, acrylic paint, and radiocontrast agent

Source of blood	Vol. of mixture (ml)	Mixture proportions			PCR inhibition ^a	DNA yield (ng/μl)	STR typing (x/y)
		Blood (%)	Acrylic paint (%)	Contrast agent (%)			
Person 1	5	50	25	25	Yes	6.64	17/17
	5	33	33	33	Yes	21.62	17/17
Person 2	5	50	25	25	No	6.45	17/17
	5	33	33	33	Yes	14	17/17
Person 1	10	50	25	25	Yes	15.44	17/17
	10	33	33	33	Yes	15.95	17/17
Person 2	10	50	25	25	No	9.07	17/17
	10	33	33	33	No	9.06	17/17

x/y (x: successfully typed STR systems; y: total of typed STR systems)

^a Yes, if difference from obliged value was >1 C_q

comprising various proportions of blood of one of two donors, acrylic paint, and radiocontrast agent. Again, PCR inhibition was observed in several samples, but all samples yielded quantifiable DNA that produced full STR profiles. These results show that neither high amounts of acrylic paint nor mixtures of acrylic paint and radiocontrast agent even in dried samples compromise DNA quality to a degree that jeopardizes STR typing.

STR typing success rate was then assessed for ten samples of backspatter recovered from swabbings of the posterior and anterior ends of the barrels of several different types and brands of firearms used to deliver contact shots at ballistic models that were doped with the triplex mixture. Table 2 summarizes these results. While DNA yield varied considerably, full STR profiles were obtained for all tested samples, indicating that neither the triplex mixture nor the physicochemical strain of the gunfire had any negative effect on STR typing success rate.

Table 2 STR typing from samples generated by experimental shots using triple mixture

Shot no.	Type	Cal.	Barrel part	DNA yield (ng/μl)	STR typing (x/y) ^a
1	Revolver	38 special	p	0.1067	17/17
2	Revolver	357 Magnum	a	0.04213	17/17
3	Revolver	38 special	p	0.4919	17/17
4	Revolver	357 Magnum	a	2.348	17/17
5	Revolver	357 Magnum	p	0.1693	17/17
6	Revolver	9 mm×19	a	0.3754	17/17
7	Pistol	9 mm×19	p	0.9175	17/17
8	Pistol	9 mm×19	a	2.514	17/17
9	Pistol	9 mm×19	a	0.04668	17/17
10	Pistol	9 mm×19	p	0.1993	17/17

Type weapon type, Cal calibre, Barrel part part of the barrel whence the sample was taken, p posterior part, a anterior part

^a x/y (x: successfully typed STR systems; y: total of typed STR systems)

Visual results

In all contact shots to the plastic bottles the gloved hand of the shooter and the weapon showed macroscopical backspatter (Fig. 2). In all tested revolvers and pistols, endoscopic inspection revealed coloured stains inside the barrel. Regularly, the intensity of stains was decreasing from the anterior to the posterior part of the barrel. The morphology of stains varied from fine droplets, elliptical and elongated forms, long or reticular spatters to blots (Fig. 3).

Radiological imaging showed highly contrasted bullet paths (Figs. 1 and 4). The cross sectional analysis of the gelatine core in 1-cm-thick slices documented the tears left by the small temporary cavity contrasted by soot which overlaid the acrylic paint (Supplementary Figure 5). Already 24 h after shooting tests, a red diffusion of blood into gelatine could be observed. However, this phenomenon did not compromise imaging.

**Fig. 2** Emulation of backspatter on the shooter's hand and the weapon

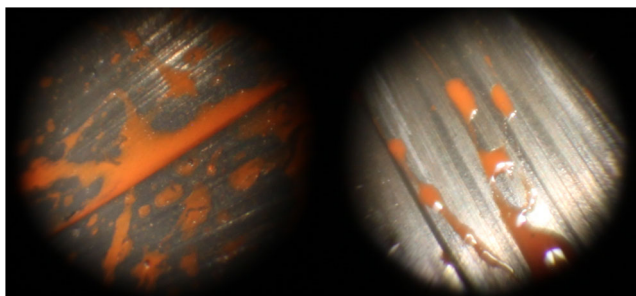


Fig. 3 Endoscopic views of barrel inside surfaces. *Left and right panels:* barrels of different revolvers with visible droplets of acrylic paint

Discussion

Backspatter on the hands of a deceased person can serve as valuable evidence of self-inflicted wounds in the investigation of gunshot injury. Obviously, the observations on backspatter reported so far are very heterogeneous [13], and backspatter analysis is regularly described in case reports [21–24].

Several different experimental approaches for the systematic investigation of backspatter have been introduced: Karger et al. shot at livestock destined for slaughter and mapped the backspatter in the surroundings of the shooter [25–27]. Radford compared the results of contact shots to severed pig heads and live, anesthetized pigs [28]. Animal experiments, however, are complex and of only limited informative value because of considerable differences between animal and human anatomy especially concerning the head.

In contrast, wound ballistic models exhibit higher reproducibility: Taylor et al. [29] and Kunz et al. [30] used blood soaked sponges in their models to emulate backspatter. Radford covered a spongy layer with artificial skin which was fixed on a gelatine block. The spongy material was injected with 20 ml of sample blood shortly before shooting [28]. Our own experimental experience indicated that thin bags made of plastic foil that are filled with blood are easier

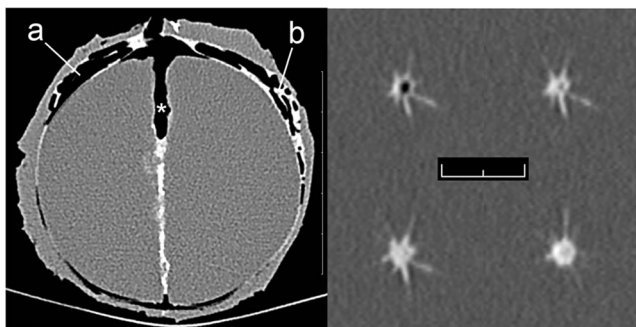


Fig. 4 CT imaging of the bullet path in the targets. *Left panel:* axial section across a plastic bottle. The entry of the bullet is on top. The silicone layer is detached by muzzle gas pressure (a) and radiocontrast agent (b) is spread outside the foil bag. The first part of the *bullet track* (*) shows major destruction of gelatine due to muzzle gas expansion. *Right panel:* Four coronal slices of the bullet track with contrasted tears (scale 1 cm)

to handle and allowed for reproducible results. Preliminary work suggested, however, that purpose-made head models outperform the classic but simplistic ballistic gelatine block models in emulating the generation of backspatter [18]. Briefly, thin, blood-containing plastic bags were glued onto a hollow acrylic sphere the surface of which was completely covered with a 3-mm layer of silicone to simulate the scalp. Contact shots to these spheres filled with ballistic gelatine were shown to produce STR-typable DNA containing backspatter in the firearms' barrel [18].

Besides backspatter collected from the shooter's hand, the presence of biological material inside the barrel is of special interest. Stone observed blood within the barrels of about 53 % of the revolvers, and 57 % of the pistols that had been used in cases of suicide [8]. Using a borescope, Visser detected blood on inside surfaces of 70 % of the examined weapons from suicide cases which was confirmed chemically [9]. The combination of endoscopic inspection of the firearm's barrel and DNA-analysis of swabbed samples of biological material collected from inner surfaces of the gun performed even better in the detection of backspatter [11]. However, small numbers of cases of gunshot suicide preclude comparative studies.

Backspatter therefore remains as a subject for experimental research. According to the principle, 'ex nihilo nihil fit', we here propose a new and integrated research concept to investigate and support the understanding of backspatter by reporting on experiments to document the formation of backspatter inside and outside the weapon as well as the projectile mediated processes inside the target. To realize this concept, the triple contrast method was developed, comprising

1. Acrylic paint for optical detection and gelatine evaluation
2. Barium sulphate as radiocontrast agent in computed tomography, and
3. Human blood for STR-based DNA typing to detect macroscopically invisible traces

Whereas acrylic paint facilitates the optical detection of backspatter, DNA typing allows detecting invisible backspatter. Simultaneously, wound ballistic analysis of the targets to evaluate the temporary cavity can be performed using the embedded radio-colour contrast in order to investigate the influence of the temporary cavity on the extent of backspatter.

Preliminary experiments proved that DNA in mixtures of different proportions of acrylic paint and/or barium sulphate with blood was fully typable using STR-PCR. For all ten contact shots, then, fired from common 9-mm weapons, backspatter on the shooter's hands and endoscopically visible traces within barrels were observed, while STR analysis resulted in full DNA profiles for all tested samples. This can be

regarded as a proof of principle. Real contact shots to the head however show less visible traces which will be investigated in further studies adjusting the head model.

It could be criticized that the physical properties and flow characteristics like dynamic and shear viscosity of our mixture may differ from those of proper blood. Notably, however, the viscosity of the triple mixture is astonishingly low when compared to the pasty acrylic paint. This might explain the successful emulation of backspatter in- and outside the weapon.

The radiological examination demonstrated a contrasted bullet path and three dimensional reconstructions were unproblematic. The cracks left by the temporary cavity were visible but short corresponding to the nondeforming bullet and the short bullet track (narrow channel). This mode of imaging could be regarded as redundant as semi-transparent models were used in the present study. Nevertheless, radiological imaging will be indispensable for nondestructive examination of non-transparent complex head models.

Finally, the gelatine core was removed from the plastic bottle and cut into 1-cm slices perpendicular to the projectile path. The amount of acrylic paint sucked into the gelatine was relatively small and in accordance with a minimal temporary cavitation of the bullet track corresponding to the narrow channel. Nevertheless, the cracks and fissures in the gelatine were well contrasted by paint and soot (gunshot residues). Already 24 h after shooting, we observed a diffusion of blood into gelatine which however did not have negative influence on imaging or the optical detectability of cracks. Even if stored at 4 °C after shooting, gelatine quickly begins to degrade and admixture of biological material like blood will usually accelerate this process. To our surprise, however, the gelatine slices remained stable for at least 5 days after shooting. In previous work, a certain conserving effect of acrylic paint had been described which may be enhanced and complemented by the antimicrobial properties of the barium sulphate component in the triple mixture.

Conclusion

We propose a new research concept to support the understanding of the formation of backspatter from shots at biological targets that aims to detect and document all traces inside and outside the weapon as well as their origin in the target. Thus, we are looking on both sides of the same coin: backspatter are traces for which we are looking for in practical work, destruction in tissue belongs to the field of wound ballistics which can help to explain the origin, respectively, the yield of such traces. Analysing both in the same shot, we document the bullet simulat interaction, and we observe the traces produced outside and inside the weapon. This approach is based on a new technique that we dubbed ‘triple contrast method’. We show that acrylic paint, enabling optical detection, and

barium sulphate, as radiocontrast agent for computed tomography imaging, can be admixed with blood without detrimental effects on the STR-typing success rate of human DNA. The triple contrast method thus allows for wound ballistical visualization and nondestructive imaging with simultaneous analysis of DNA contained in backspatter.

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Conflict of interests The authors declare that they have no conflict of interests.

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