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On the effect of shooting distance, ballistic model construction, doping and weapon type on the simultaneous analysis of DNA and RNA from backspatter recovered from inside surfaces of firearms

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ABSTRACT

Investigations at crime scenes after criminal acts involving gunshot injuries have occurred often encompass the analysis of traces of blood and so-called backspatter. Molecular genetic analysis of backspatter generated by contact shots and shots from very short distances has already been demonstrated to critically contribute to victim identification and the reconstruction of firearm-related crimes.

Herein, we investigated the effect of several combinations of shooting distances and types of firearms on backspatter generation and co-extraction and simultaneous analysis of DNA and RNA isolated from traces of backspatter. Additionally, we assessed whether 'triple contrast' doping of ballistic models interferes with forensic analysis of DNA, mtDNA and co-extracted mRNA and miRNA from backspatter collected from inside parts of firearms generated by experimental shootings.

We show the effect of shooting distance and the type of firearm in experimental shootings on the yields of DNA and RNA co-extracted from backspatter and the success rates of forensic DNA profiling and RNA based organ identification. Furthermore, we demonstrate that 'triple contrast' stained biological samples collected from inside surfaces of firearms are amenable to forensic DNA profiling and permit analysis of the entire mtDNA D-loop even for 'low template' DNA amounts that preclude standard short tandem repeat DNA analysis.

1. Introduction

The analysis of bloodstain patterns is an important aspect of forensic crime scene reconstruction. Caused by shots against biological targets a spray of biological material (e.g. blood and tissue) can be ejected from the entrance wound and be propelled back into the direction of the firearm ('backspatter'). Traces of backspatter may consolidate on and be recovered from the shooter and the shooter's surroundings but also from inside surfaces of the firearm. However, such criminal acts involving shooting firearms at biological targets cannot be planned or controlled, therefore experimental shootings have to rely on standardized ballistic models [1,2] and recently, a new modelling method was presented that allows for the economic molecular analysis of DNA from backspatter with simultaneous investigation of wound ballistic phenomena [3]. Herein, we aim to extend this method's versatility to include the analysis of mtDNA and RNA (project B).

Another very important aspect in the evidence based reconstruction and legal appraisal of firearm related crimes is the distance from which a shot has been fired. Therefore, we present a systematic investigation of the effect of shooting distances up to 30 cm on the molecular biological analysis of nucleic acids from traces of backspatter recovered from inside surfaces of firearms as it is currently assumed, that backspatter is recoverable only after close range or contact shots (project A).

2. Material and methods

Acquisition of samples, doping and construction of ballistic models were performed as described elsewhere [3–5]. For experimental shootings, sampling procedure [6], RNA/DNA co-extraction, quantification, integrity assessment and STR profiling, reverse transcription (RT) and qPCR see Fig. 1. Analysis of mitochondrial DNA e.g. procedures for target amplification, sequencing and electrophoresis are described in previous work [4,7] and (Fig. 1).

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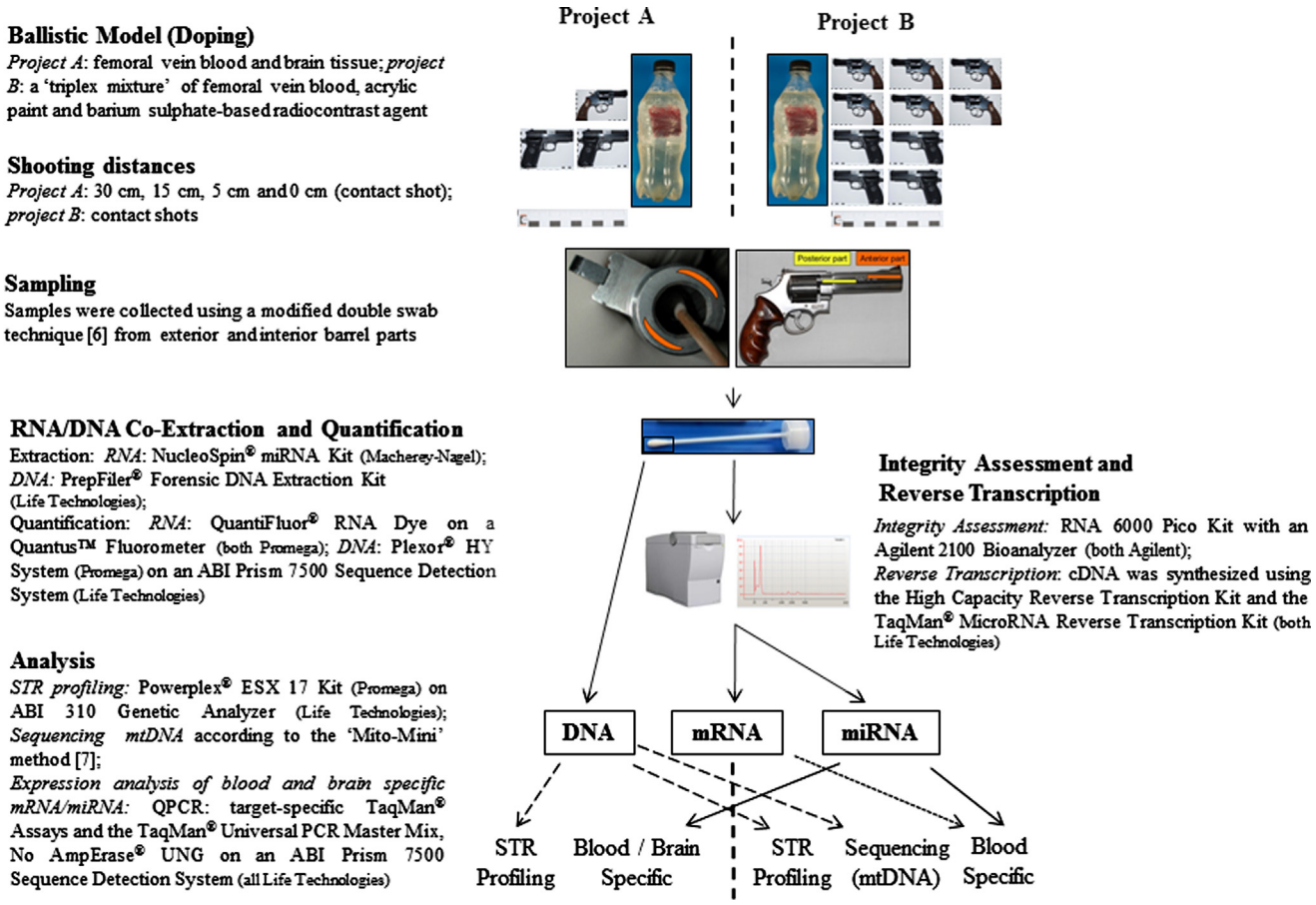

Fig. 1. Overview of workflow of project A and/or B.

2.1. Selection of specific mRNA/miRNA and reference genes for qPCR data normalization

The following blood and brain specific mRNA and miRNA were chosen based on previous work [8–10]: β-hemoglobin (*HBB*) and *miR-16* for blood and miR-124a for brain tissue. Selection of candidate reference genes was performed as described elsewhere [4,5]. Briefly, we chose ribosomal protein L37a (RPL37A) and *SNORA66*/m *iR-191* as the reference genes best suited for blood and brain tissue specific mRNA and miRNA expression data normalization, respectively.

2.2. Data analysis

Data was analysed, normalized and processed as described elsewhere [4,5,11]. A normalized C_q-value of <35 was considered to indicate successful PCR implying a target specific signal and thus RNA quantity and quality suitable for expression analysis.

3. Results

3.1. Quantification and STR profiling of co-extracted DNA

After shots had been fired at ballistic models from specified distances, backspatter containing biological material was collected from various sampling locations as described in [4,5]. Positive DNA quantification results were obtained for all collected samples with variance of DNA yields between samples from different barrel parts, weapon types and blood donors. To assess the success rate of STR typing from distance shots and 'triple mixture' stained samples as limited by DNA amount, DNA profiles were generated for selected samples with varying DNA yields [4,5]. Full STR profiles (17 of 17 possible STR systems) were obtained for all samples with an STR-PCR input amount of >100 pg of DNA,

3.2. Analysis of mtDNA collected from 'triple mixture' doped ballistic models

All amplified fragments, representing the entire D-loop region of mtDNA, were sequenced for selected samples that exhibited a DNA amount less than 100 pg and for which only partial or no STR profiles at all had been obtained. For all tested samples, at least 8 of 10 fragments could successfully be analysed.

3.3. Expression analysis of blood and brain specific mRNA/miRNA

To assess the general suitability for forensically relevant downstream analyses of RNA isolated from backspatter collected from inside surfaces of weapons after shots on ballistic models from various distances or doped with 'triple mixture', respectively, expression levels of blood and brain specific mRNA (*HBB*) and miRNAs *miR-16*/*miR-124a*, respectively, were determined by qPCR in selected samples. Overall, normalized expression of mRNA *HBB* was blood specific ($C_{q,n}<35$) in all selected samples. Blood specific

normalized expression of *miR-16* ($C_{q,n}$ <35) was detected in all selected samples. Also, presence of blood was correctly called in samples generated from all shooting distances up to 30 cm. Notably however, detection of brain proved less sensitive as brain specific normalized expression levels of *miR-124a* ($C_{q,n}$<35) was obtained only in a half of selected samples and only for shooting distances up to 15 cm or contact shots (=0 cm).

4. Conclusion

Backspattered material from blood and/or brain tissue was successfully recovered and analyzable with shooting distances of up to 15 and 30 cm, respectively. These results indicate that traces of backspatter on inside surfaces of firearms should be regarded as a valuable source of forensic evidence not only in contact shots. 'Triple contrast' stained biological material collected from inside surfaces of firearms are amenable to the full bandwidth of forensic nucleic acid analysis encompassing nuclear (nDNA) and mitochondrial DNA (mtDNA), mRNA and miRNA. The analysis of mtDNA is an alternative approach to standard STR profiling if only insufficient and/or highly degraded nDNA can be retrieved.

Conflict of interest

None.

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