Forensic Science International 216 (2012) 121–126

Contents lists available at SciVerse ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

# Phantoms in the mortuary—DNA transfer during autopsies

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ARTICLE INFO

*Article history:*
Received 21 January 2011
Received in revised form 18 July 2011
Accepted 9 September 2011
Available online 2 October 2011

*Keywords:*
STR typing
Autopsy
Forensic
DNA contamination

ABSTRACT

DNA evidence frequently plays an important role in criminal investigations and in some cases may be the only means of convicting a suspect. The constant improvement of DNA analysis techniques affords the individualization of minute amounts of DNA, aggravating the risk of contamination artifacts.

In our study, we investigated the prevalence of DNA contamination in the autopsy facilities of the Institutes of Legal Medicine in Essen and Kiel (Germany). Using DNA-free swabs, we took samples from instruments used during autopsy and autopsy tables. Surfaces and instruments were routinely cleaned before sampling. Swabs were subjected to different PCRs to quantify the total amount of DNA and to amplify individual specific STR-markers. In most samples, alleles that could be linked to bodies that had been autopsied before were found. Furthermore, we could show that a DNA transfer from the autopsy table to a body was detectable in four out of six cases investigated. The interpretation of DNA typing results may thus be severely complicated. To avoid DNA contamination, we tried out different cleaning methods, of which only a bleach containing cleaner showed sufficient results.



## 1. Introduction

DNA evidence frequently plays an important role in criminal investigations and in some cases may be the only means of convicting a suspect. The constant improvements in forensic genetic analysis have led to a very low detection threshold for DNA containing traces. Recently, new multiplex kits for amplification of so-called mini-STRs were developed enabling detection of DNA amounts of 25 pg and less [1–4]. Nowadays, successful DNA analysis is possible from samples previously considered unfeasible for autosomal DNA detection, e.g. telogen hairs, old bones or teeth, or minute amounts of highly degraded DNA [5]. Even DNA profiles from simple fingerprints [6,7] and ammunition [8] could be detected, and new techniques certainly will further enhance the typing success.

However, the ever-continuing improvements of forensic DNA typing aggravate the eminent problem of contamination. DNA contamination can occur at any time during a criminal (homicide-)investigation, be it at the crime scene, e.g. by the police or emergency personnel, during each handling of the body on the way to and at the morgue, and also during autopsy. As early as in the 1990s, some authors pointed out the danger of sample contamination with DNA from foreign sources, i.e. sources not linked to the crime investigated, during the post-mortem examination [9–11].

The contamination of trace materials can complicate the interpretation of DNA typing results. This may especially be true in cases where only minute amounts of perpetrator DNA are present, e.g. single epithelial cells in cases of murder or rape, or when a decomposed or severely burned body with only little amounts of original DNA needs to be genetically identified.

In our study, we investigated contamination hazards in the autopsy facilities of two German institutes of legal medicine (Essen and Kiel). The aim of our investigation was to identify contaminant sources, to evaluate the risk of secondary DNA transfer, and to establish procedures to eliminate or at least minimize the contamination risk.

## 2. Materials and methods

### 2.1. Sample collection and DNA isolation

For contamination experiments, prior to 27 autopsies (in 20 cases after routine cleaning as described under Section 2.4; in 7 cases after decontamination with bleach or DNA Exitus Plus® as described in Section 2.5) a total of 259 samples were collected using DNA-free swabs (Forensic Swab, EtO-sterilized, Sarstedt, Germany). Samples were taken from the following instruments and locations: toothed forceps (two pairs per institute; Aesculap, Tuttlingen, Germany) ($n = 56$), autopsy tables (stainless steel, Funeralia, Würzburg, Germany) ($n = 144$), measuring stick for measuring e.g. bruises or other noticeable injuries (in Essen: plastic, 50 cm long, Aesculap, Tuttlingen, Germany; Kiel: metal, 50 cm long) ($n = 23$), and neck rests (Essen: plastic; Kiel: wooden) ($n = 36$). Up to 10 swabs were taken per autopsy.

To test the possibility of DNA transfer from one body to another via the autopsy table, samples were taken from contact points between the body to be autopsied and the autopsy table (scapular region and buttocks) in six cases. Minimum contact

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doi:10.1016/j.forsciint.2011.09.006

**Table 1**
Methods tested to remove DNA contamination. The table shows the chemicals used, the tested treatments, and the respective results regarding detection of human specific DNA signals after screening PCRs.

| Cleaning procedure to remove DNA | Forceps | Tables |
|---|---|---|
| Bleach 2.8%, 30 min incubation, rinsing with water | 0 | n.t. |
| Bleach 0.28%, 30 min incubation, rinsing with water | 0 | n.t. |
| Bleach 2.8%, 15 min incubation, rinsing with water | n.t. | 0 |
| Bleach 0.28%, 15 min incubation, rinsing with water | n.t. | 0 |
| Perform® 1%, 15 min incubation, rinsing with water | n.t. | 1, 2, (3) |
| Perform® 1%, scrubbing, then 15 min incubation, rinsing with water | n.t. | 2, 3 |
| Antifekt 0.75%, 15 min incubation, rinsing with water | n.t. | 1, 2 |
| Antifekt0.75%, scrubbing, then 15 min incubation, rinsing with water | n.t. | 2, 3 |
| DNA Exitus Plus®, spraying on instruments or surfaces and incubation for atleast 15 min | n.t. | 1 |

time between body and table was 15 min. A total of 24 swabs (four per body) were taken for these DNA transfer experiments.

Additionally, swabs were taken from three different plastic canvas covers (Götz Trauerwaren, Regensburg, Germany; Pludra Frankfurt GmbH, Frankfurt, Germany) experimentally used to avoid contact between the body and the surface of the autopsy table. The covers were new and originally sealed, they had not previously been used for other bodies. Swabs ($n = 3$ per cover) were moistened with DNA-free water before sampling, the covers were extensively wiped on the inner side.

All swabs were extracted using the Invisorb Spin Swab Kit (Invitek, Berlin, Germany) and by means of Phenol-Chloroform extraction (Kiel and Essen, respectively).

Every body examined in this study and those bodies considered possible contaminants (i.e. bodies that were not investigated in this study but were autopsied during the study period on the tables investigated) ($n = 42$) were genetically typed as described below.

### 2.2. Genetic analysis of isolated DNA and fragment analysis

For contamination experiments, an aliquot of each sample (1 μl) was employed to multiplex PCRs using the Powerplex® S5 or Powerplex® ESX17 kits (Promega, Germany) in Essen (PP S5 $n = 90$; PP ESX $n = 40$) or a screening PCR especially designed for minute amounts of DNA and degraded DNA samples [4] in Kiel ($n = 129$). Template input of samples with low signal intensities or no signals at all was doubled exemplary ($n = 20$). In Kiel, selected samples obtained when wiping the autopsy tables were employed to amplify male specific STRs using the Powerplex® Y kit (Promega, Germany) ($n = 10$).

For fragment analysis, 0.2 μl per sample of the required size marker was used (Powerplex® ESX: ILS 500, Powerplex® S5 and Powerplex® Y: ILS 600 [both Promega]; screening PCR amplicons: ROX500 [Applied Biosystems]). Electrophoresis was performed on an ABIPrism® Genetic Analyzer 310 (Essen) or 3130 (Kiel) (Applied Biosystems).

Allele assignment was performed by comparison with commercially available ladders and a self-made allelic ladder for the screening-PCR. Determination of fragment sizes was done using the 310 Gene Scan 3.1.2 software or the GeneMapper ID v3.2 (both Applied Biosystems). Peaks below 50 relative fluorescent units (rfu) were not analyzed. Identical procedures were used for transfer experiments. Bodies and plastic canvas covers were investigated using the Powerplex® ESX kit.

### 2.3. DNA quantification using real time PCR

Exemplary, DNA content of a sample subset ($n = 44$) was measured using a self-made real time PCR assay as described in Poetsch et al. [12]. Two microliters per sample were used as template, every amplification was done in triplets. Samples were randomly chosen to obtain a general overview over the DNA content with respect to STR analysis results. Quantification was not used for the optimization of DNA template input for STR analysis.

### 2.4. Routine decontamination of surfaces and instruments in the autopsy room

At the beginning of this study, the following routine cleaning procedures were applied at the respective institutes:

**Kiel**: After working hours (evenings and weekends) the whole room was UV-irradiated using UV-lamps (wave length 254 nm, energy 5 mW/cm) hanging from the ceiling. After every use, dissecting instruments were incubated for 30 min in Sekusept Forte plus Sekusept Cleaner (3%, Ecolab, Düsseldorf, Germany), a disinfectant against bacteria, fungi, and in parts against viruses. Surfaces were cleaned with Antifect FD10 (Schülke & Mayr, Norderstedt, Germany), a disinfectant for medical products that is active against bacteria, fungi, MRSA, HIV, Hepatitis-B, rota virus, and spores. When performing more than one autopsy per day, all used instruments were rinsed extensively with water and soap. The table was cleaned in the same way until all visible staining was gone and subsequently cleaned with Antifect. The whole dissecting room was cleaned thoroughly by treating all surfaces including floor and tiles with Antifect weekly.

**Essen**: After working hours (evenings and weekends) instruments, autopsy table and floor were rinsed with clear water first and after removal of coarse contamination (e.g. blood, tissue or fatty streaks) cleaned with a commercially available dish-soap. Afterwards, the surfaces and instruments were treated with a disinfectant based on active oxygen (Perform®, Schülke & Mayr, Norderstedt, Germany) according to the manufacturers guidelines in a concentration of 1%. Perform® is active against bacteria (incl. TB), fungi, viruses (Hepatits B, HIV, rota, adeno, polio, vaccinia, and the SV40 papova virus), and spores. It is especially designed for the use on all types of surfaces and is suitable for use in medical risk areas. Before weekends, the whole dissecting room was cleaned thoroughly by treating all surfaces including floor and tiles with Perform®. When doing more than one autopsy per day, all used instruments and the autopsy table were only rinsed with water and a commercially available dish-soap to remove visible coarse contaminations.

### 2.5. Experimental setup for decontamination of surfaces and instruments to remove DNA traces and precautions to avoid contaminations

Two different procedures were tested to remove DNA prior to the next autopsy:

Cleaning with bleach (DAN Klorix®, Colgate Palmolive, Vienna, Austria): Instruments were soaked for at least 30 min in two differently concentrated solutions (pure: 2.8% and diluted: 0.28%); the table was rinsed and scrubbed with bleach and soaked for at least 15 min. The remaining bleach was washed up with tap water. Each concentration was tested prior to four different autopsies.

Prior to three autopsies, the tables were also cleaned in the same way using a combination of Antifect FD 10 (Kiel) or Perform® (Essen) (both Schülke & Mayr, Norderstedt, Germany) and DNA Exitus Plus® (AppliChem, Darmstadt, Germany). See Table 1 for an overview.

## 3. Results and discussion

### 3.1. DNA yield from different samples

According to real time PCR results, DNA concentrations from none to 4.2 ng/μl were obtained, with DNA yields of up to 4.2 ng/μl for forceps, 2.1 ng/μl for autopsy tables, 0.55 ng/μl for

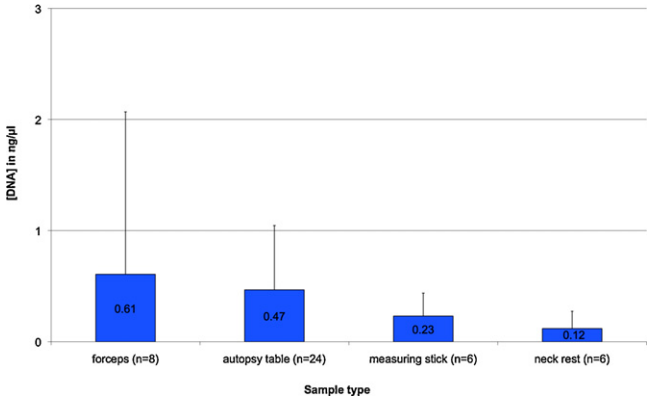


**Fig. 1.** DNA content of samples taken in the autopsy suite after routine cleaning procedures. Schematic presentation of real time PCR results. Shown are the average values and the corresponding standard deviations for DNA amounts in ng per μl in samples obtained from different instruments. Maximum value (for forceps) was 4.2 ng/μl (see main text for additional information).