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Research paper

Contamination incidents in the pre-analytical phase of forensic DNA analysis in Austria—Statistics of 17 years

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ABSTRACT

Forensic DNA analyses have become more and more sensitive in the past years. With the ability to generate DNA profiles even from minute amounts of cellular material also the possibility to detect DNA on trace material that originates from persons not linked to the crime event, such as crime scene investigators, increases. The contamination of crime scene samples can lead to false positive results and misinterpretation that can cause deceptive investigations. In this work we continue a study of 2010 that compared the number of detected contamination incidents that were caused in the pre-analytical phase of forensic DNA analysis with the number of crime scene samples analyzed by our laboratory. Within the past 17 years we were able to detect a total of 347 contamination incidents caused by police officers in approximately 46,000 trace samples to their origin (0.75%). Additionally we demonstrate the usefulness of reference profile databases that contain DNA profiles of police officers to detect contamination incidents of trace material.

1. Introduction

The assignment of biological material on exhibits of a crime scene to its origin often provides crucial evidence in judicial proceedings. Modern DNA typing techniques significantly simplified creating a DNA profile out of only few cells on evidence material, as the sensitivity of DNA analysis methods was steadily increased. Thereby also the chance to detect minute amounts of biological material that do not originate from a person involved in the crime event was enhanced. This in turn is prone to misleading DNA evidence due to misinterpretation and false positive results regarding contamination incidents [1]. This was clearly shown during the search of an unknown female person also known as 'Phantom of Heilbronn'. A female DNA profile found on evidence material of more than 40 crime scenes in Germany, Austria and France including a homicide of a police officer in 2007. As solved in 2009, the female DNA profile originated from an employee of a manufacturer of the evidence collection swabs [2,3]. Additionally to false positive results that can lay a false trail, DNA traces of the perpetrator can also be hidden by the extraneous component leading to false exclusions.

Contamination, which means the transfer of DNA after the crime event [4] basically can occur at any point of two different stages during forensic DNA analysis:

- the pre-submission or pre-analytical phase that involves the location, recovery, packing as well as storage and transportation of evidence material; and
- the analytical phase that involves all laboratory working steps of the DNA analysis process [5,6]

Possible sources of DNA contaminations are:

- during manufacturing contaminated consumables
- investigative agencies and other persons present at the crime scene (e.g. police officers, rescue workers)
- laboratory workers
- cross contamination (from exhibit to exhibit or sample to sample) [4,5]

In criminal proceedings not only the origin of biological traces is of interest, but also the way the biological material was transferred to the evidence material [7]. Biological material can either be transferred by means of direct or indirect transfer. Direct transfer, also called primary transfer, not only includes direct contact such as touching but also activities in close vicinity of an item (e.g.

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coughing, speaking, sneezing). Indirect or secondary transfer means the transfer of DNA via several steps (e.g. from an individual to an object via touching and then from this touched object to another object) [1,4,8,9].

As the contamination of crime scene samples still presents a problem in forensic DNA analysis, the prevention as well as the optimization of the identification process of contamination incidents is of particular importance.

The detection of contamination incidents can be significantly enhanced with the availability of DNA elimination databases (EDB) containing reference profiles as recommended by the European Network of Forensic Science Institutes (ENFSI) [1,5,10,11].

Since 2009 Austria's national DNA database is running an associated Police Elimination Database (PED) containing DNA profiles of police officers. In addition to the national PED our laboratory that mainly is analyzing evidence material from the federal states of Salzburg and Upper Austria has another internal EDB. The Profile Comparison tool of the GeneMapper™ ID-X software (Life Technologies) has been used since 2013 to exclude all institute members, cleaning and maintenance staff as well as all crime scene investigators from Salzburg and Upper Austria prior to submitting a profile to the national DNA database.

In the present study we compare the number of detected contamination incidents caused by police officers in the pre-analytical phase of forensic DNA analysis with the number of crime scene samples analyzed by our laboratory. Thereby we are continuing a former study from 2010 [12].

2. Materials and methods

2.1. Sample preparation and analysis

Within the years 2000 to 2016 the DNA of approximately 46,000 crime scene samples from the federal states of Salzburg and Upper Austria was extracted using phenol/chloroform extraction, the "First-DNA" all tissue kit (GEN-IAL) or a M48 robot (Qiagen). Following extraction, DNA was amplified on GeneAmp 9700 PCR Systems (Applied Biosystems) using 28 to 30 cycles and at least one of the following DNA amplification kits: AmpflSTR SGM Plus, SEFiler Plus, NGM Select (Life Technologies), PowerPlex ESX 17, ESI 17 (Promega), AmpflSTR Yfiler or AmpflSTR Yfiler PLUS (Life Technologies). Electropherograms were generated by capillary electrophoresis of 0.5 to 5 μl of amplification products (based on comparison to a reference sample of known concentration using polyacrylamide gel electrophoresis) in 25 μl of HiDi formamide/size standard mix on ABI 310, ABI 3100 AVANT, or ABI 3500 Genetic Analyzers (Applied Biosystems) and analyzed using Genotyper v3.7 and GeneMapper™ ID-X v1.4 (Life Technologies), respectively.

2.2. Detection of contamination incidents

At the present time our laboratory is detecting contamination incidents by combining a manual check with database assisted profile comparisons using the national Police Elimination Database (PED) as well as the profile comparison tool of the GeneMapper™ ID-X software.

2.2.1. Since 2000: manual profile comparison

With support of the responsible chief officers of the state offices of criminal investigations (LKA) from the federal states of Salzburg and Upper Austria our laboratory has been collecting reference samples from crime scene investigators since 2000. The generated reference DNA profiles are then used for manual screening for contamination incidents. As the manual profile comparison is very time consuming, the DNA profiles found on the trace material are only compared with the reference profile of the relevant crime scene investigator that has collected the evidence material.

2.2.2. Since 2009: Police Elimination Database (PED)

The Austrian Police Elimination Database (PED) is integrated in the national DNA database and operated by the Austrian Federal Ministry of Interior (BMI). It includes DNA profiles as well as dactylograms of police officers and crime scene investigators for elimination purposes. These DNA profiles are not used for synchronization with other DNA databases and are stored anonymized using a barcode system. The identity of the corresponding police officers is only known by the Ministry of Interior and two executives of the responsible state office of criminal investigations. Following a pilot project between 2008 and 2009, the Police Elimination Database has been used since October 2009 to identify contamination incidents during crime scene investigation. In Austria, the registration of DNA profiles of crime scene investigators is mandatory, whereas the acquisition of data of all other police officers occurs on a voluntary basis. The record of personal data for identification purposes is regulated in the security police act ("*Sicherheitspolizeigesetz*"). By the end of 2016, a total of 4654 DNA profiles of police officers were registered in the PED. Following a match, the forensic laboratories as well as the responsible executives of the state office of criminal investigations are informed by the BMI. The executives of the state office of criminal investigations then check whether the police officer that caused the contamination was involved in that particular case and therefore authorized to be at the crime scene or not. Afterwards they will inform the police officer whose DNA traces were found and ask whether there are any ideas on how the contamination could have occurred.

If the contamination incident caused a simple profile isolated from the crime scene sample the DNA profile will be excluded from the national DNA database following identification of the contamination. Since 2017, also mixed DNA profiles are automatically checked for contamination incidents. If a mixed profile with unknown contributor's DNA is found, the DNA profile remains in the national DNA database for further alignment.

2.2.3. Since 2013: GeneMapper™ Profile Comparison tool

The GeneMapper™ ID-X software (Life Technologies) contains a feature for QC/QA checks that can help to identify contamination incidents – the so-called "Profile Comparison" tool. The Profile Comparison tool includes an internal database with the option to upload custom samples and controls for automatic concordance checks and allele matching [13]. With the possibility to set an individual match percent threshold (meaning the percent of reference profile alleles detected in the comparison profile), the detection of contamination incidents can be further enhanced. Especially in cases of complex mixtures this tool can help to reduce false positive DNA profiles. In our laboratory DNA profiles of 526 crime scene investigators of the federal states of Salzburg and Upper Austria as well as profiles from all our institute members are included in the in-house reference database of the GeneMapper™ ID-X Profile Comparison tool to perform an automatic contamination check before submitting a trace sample DNA profile to the national DNA database.

3. Results

3.1. Contamination statistics

3.1.1. 2000–2009: manual screening

Since the year 2000 our laboratory has been using DNA profile tables of police officers to check DNA profiles generated from trace material for contamination incidents. During 2000 and 2009, a