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Case reports

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Filter mask as a new candidate of personal belonging used in cadaver identification – a case report

Maseczka na twarz jako nowy kandydat wśród rzeczy użytku osobistego w identyfikacji zwłok – opis przypadku

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Abstract

The case report presents an identification process based on DNA isolated from personal belongings, including a filter mask. In May 2021, an unidentified 65-year-old male corpse was revealed by the city's outskirts road. Since it was impossible to use material from living relatives for comparative analysis, the samples of personal belongings of the alleged victim were used instead: clippings of the filtering face piece type 2 (FFP2) face mask (parts adhering to the nose and the earlobes, the central part of the mask), swabs from the razor (blade and shaft), toothbrush shaft, and toothbrush filaments clippings. The presented case indicates the need for collecting a wide range of samples for genetic analyses, including filter masks as an alternative item of personal belonging.

Key words: filter mask, genetic analyses, personal belongings

Streszczenie

Zaprezentowany opis przypadku przedstawia próbę identyfikacji osobniczej z wykorzystaniem DNA wyizolowanego z rzeczy użytku osobistego, w tym maseczki na twarz. W maju 2021 r. przy drodze podmiejskiej znaleziono niezidentyfikowane zwłoki 65-letniego mężczyzny. Ponieważ niemożliwe było wykorzystanie do analizy porównawczej materiału od żyjących krewnych, wykorzystano rzeczy osobiste domniemanej ofiary: wycinki maski filtrującej typu 2 (FFP2) (części przylegające do nosa i małżowiny usznych, środkową część maski), wymazy z maszynki do golenia (ostrze i trzonek), wymaz z trzonka szczoteczki do zębów i wycinki z włosia szczoteczki. Przedstawiony przypadek wskazuje na potrzebę pobrania szerokiego zakresu próbek do analiz genetycznych, w tym masek filtrujących jako alternatywnego przedmiotu użytku osobistego.

Słowa kluczowe: maseczka na twarz, analizy genetyczne, przedmioty użytku osobistego

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1. Introduction

The Interpol Disaster Victim Identification Guide recommends DNA profiling and matching as one of the primary methods to identify missing persons during a disaster [1]. However, in some cases, biological material from relatives is difficult or impossible to obtain. Good sources of DNA that can be used in the identification process recommended by Interpol are razor blades and toothbrushes [2].

During the SARS-CoV-2 pandemic, it is recommended to use masks in most countries. Filter masks (such as N95 (USA), KN95 (China)), are mandatory in many countries as they protect from unintended exposure to viruses, e.g., influenza virus or coronavirus [3]. N95/FFP2 masks are high-filtration, negative-pressure respiratory masks that filter at least 94–95% of 0.3–0.6 μm aerosol particles [4] and 98.8–99.8% of 0.04–150 μm aerosol particles [5]. The meta-analysis of 12 primary studies demonstrated that using a filter mask significantly reduces the risk of transmitting respiratory infections [6].

However, the healthcare workers wearing filter mask longer than 4 hours complained about sweating around the mouth which resulted in poorer adherence and in-creased the risk of infection [7]. We hypothesized that the mask material could serve as a DNA source due to the close contact with the face.

The presented case report shows the usefulness of filter masks in forensic analyses and compares the efficacy of DNA isolation from different parts of different personal belongings.

2. Case description

The corpse of a 65-year-old man was revealed by the road on the city's outskirts in May 2021. According to the Prosecutor's Office information, supposedly, the man set himself on fire after being doused with a flammable petroleum derivative. The inspection of the incident site revealed the corpse lying on the lawn. Due to extended body scorching, it was impossible to evaluate the lividity. Rigor mortis was present in all muscle groups. The limbs were in flexion position, bilateral, with high degree contracture in the elbow and hip joints.

Based on the identity documents found at the incident site, it was possible to establish the victim's alleged identity. The testimony of the witnesses who saw the alleged victim for the last time allowed to set the time from death to the start of the autopsy at four days. The reason for this act was most likely due to family problems.

2.1. Autopsy findings

The forensic report stated features of a thermal flame burn (of the 2nd and the 2nd/3rd degree) practically on the body's entire surface (Fig. 1a) and the respiratory tract. It also stated thermal brain damage, deep soot aspiration into the larynx, tra-

Fig. 1. Collecting biological material during autopsy: a) postmortem image of a self-immolated cadaver; b) costal cartilage samples before (left side) and after cleaning from muscle tissue (right side)

chea, bronchi, esophagus, and stomach, pulmonary edema and congestion (with numerous subpleural hemorrhages presence), and bright red discoloration of lividity, blood, and muscles on the cross-sections.

Toxicological analysis showed no ethyl alcohol in the blood and urine of the deceased. The material sampled from the brain and lungs showed the presence of aliphatic hydro-carbons. The blood contained 23% of carboxyhemoglobin, and although such concentration does not prove a fatal carboxyhemoglobin poisoning, it may additionally indicate the intentional use of the flame.

Body inspection, autopsy, and the results of analyzes mentioned above indicated that the cause of death of the victim was an extensive thermal injury (burn of the body) in the course of flame action. According to the information obtained from the Prosecutor's Office regarding the circumstances of the event, it seemed probable that the above-mentioned thermal injury was a consequence of self-ignition after being doused with a flammable petroleum derivative, like gasoline or similar. It was evidenced by the presence of aliphatic hydrocarbons found in the lungs and brain of the deceased.

Due to the extensive thermal damage of the body (Fig. 1a), the facial characteristics and fingerprints could not be used in the identification process. The investigation conducted by police officers revealed that it is impossible to obtain comparative material from family members of the alleged victim. Therefore, the autopsy material was secured to extract the DNA profile of the cadaver, and the prosecutor decided to conduct a comparative analysis of the DNA extracted from the section material and the DNA isolated from the personal belongings of the alleged victim.

2.2. Material and Methods

DNA profiling, commissioned by the Prosecutor's Office, included using postmortem samples col-

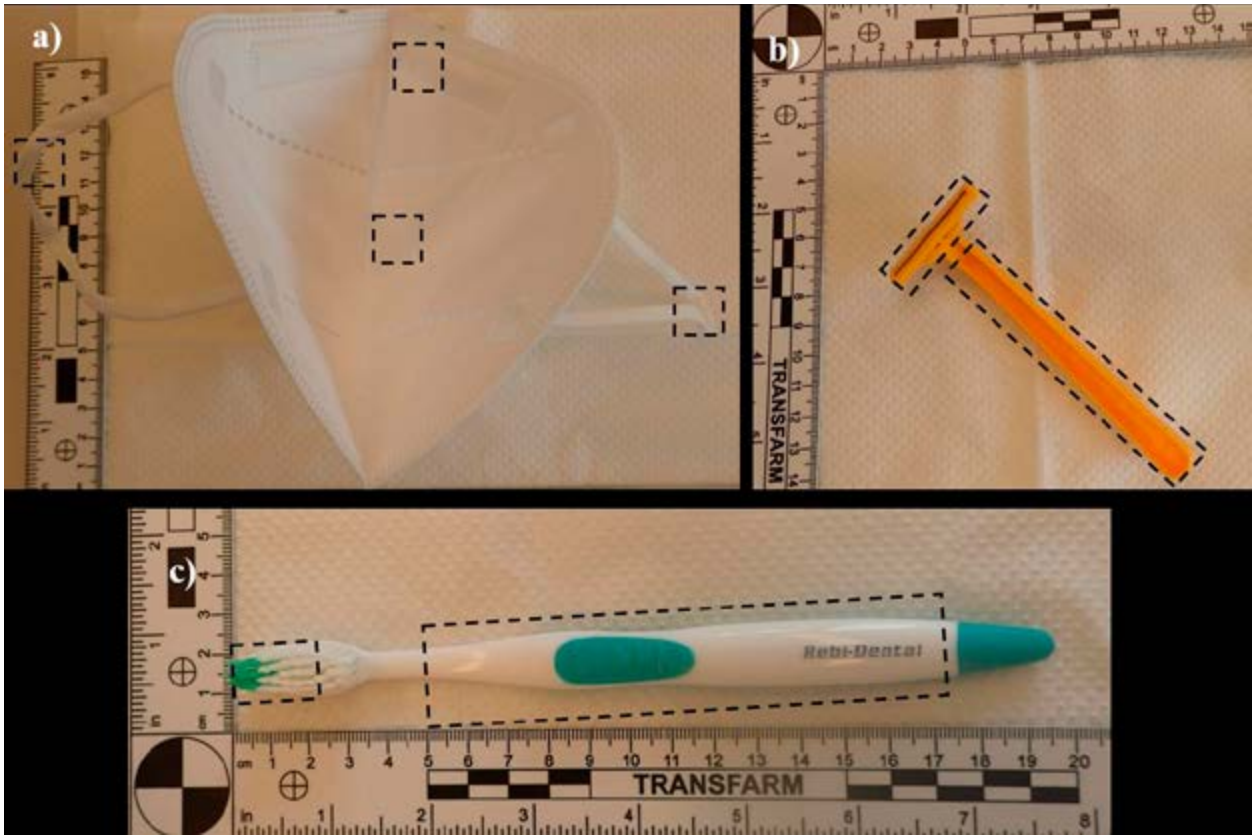

Fig. 2. Personal belongings secured for the DNA identification process of the alleged victim: a) filter mask, b) razor, c) toothbrush. The dotted lines denote areas of DNA sampling: a) clipped areas, b) swabbed area, c) clipped (toothbrush head), and swabbed (toothbrush shaft) area

lected from the cadaver and antemortem samples collected from the alleged victim's apartment.

Since, the medical documentation was missing in the described case, the blood transfusion or a bone marrow transplant status of the cadaver was unknown. Therefore, costal cartilage (cc) was selected as a source of postmortem samples for the genetic analysis, as this tissue is free from the potential effect of chimerism [8]. Costal cartilage fragments were collected and prepared using the method described earlier [9]. Briefly, a 4 × 7 cm fragment of costal cartilage (Fig.1b) was collected during forensic autopsy from the rib arch of the cadaver. Its external surface and contaminations were cleaned. Then the sample was fragmented into slices using a sterile scalpel blade (Fig. 1b). Next, 80 mg of fragmented cartilage were placed in 1.5 ml Eppendorf tubes and incubated in an extraction mixture (300 µl ultrapure H_2O, 300 µl lysis buffer L 1.4, and 20 µl proteinase K (Sherlock AX kit, A&A Biotechnology, Poland)) at 50°C and shaken in a thermomixer at 400 rpm for 6 h. DNA isolation was performed using a Sherlock AX kit (A&A Biotechnology, Poland). The final volume of the DNA solution used was 50 µl.

Antemortem samples were collected from the alleged victim personal belongings (filter mask, razor, and toothbrush). Clippings from the filter mask were taken from parts adhering to the nose, central part, and elastic straps adhering to the earlobes. Swabs from the razor were taken from the blades and the shaft. Samples from the toothbrush were pre-pared from the swabs taken from the shaft part (swab) and 40 filaments clipped from the toothbrush head (Fig. 2).

DNA was isolated using the Sherlock AX kit (A&A Biotechnology, Poland). Samples were incubated for 1 h. The final volume of the DNA solution used was 20 µl.

2.2.1. Real – Time PCR

The quality and concentration of DNA samples were evaluated using a QuantifilerTM Trio DNA Quantification kit (Applied Biosystems, USA), HiD v. 1.2 software, and Applied Biosystems™ 7500 Real-Time PCR System (Applied Biosystems, USA). The qualitative analysis was performed using sequences for T. Large (214 bp) and T. Small (80 bp) autosomal chromosome, and the Y chromosome (75 bp). The degradation index was calculated based on the ratio of T. Small to T. Large autosomal sequences.

2.2.2. Multiplex PCR and capillary electrophoresis

PCR reaction was performed using a Power Plex ESX 17 and Power Plex HS 16 kit (Promega Corporation, USA) in a Gene Amp PCR System 9700 thermocycler (Applied Biosystems, USA). Amplification products were separated towards DNA CC5 ILS 500 and CC5 ILS 600 standards (Promega Corporation, USA) using 3130 Genetic Analyzer (Applied Biosystems, USA). The following loci were analyzed: AMEL, D3S1358, TH01, D21S11, D18S51, D10S1248, D1S1656, D2S1338, D16S539, D22S1045, VWA, D8SS1179, FGA, D2S441, D12S391, D19S433, SE33, D5S818, D13S317, D7S820, TPOX, CSF1PO, Penta D and Penta E. Additionally, alleles from chromosome Y were determined using a Yfiler test (Applied Biosystems, USA). PCR products were analyzed in a 3100 Genetic Analyzer (Applied Biosystems, USA). Genotypes were generated using a Gene Mapper ID v3.2 software (Applied Biosystems, USA).

2.2.3. Statistical analysis

Likelihood ratio (LR) was calculated using DNA Stat software v. 2.1 (Laser Systemy Informatyczne S.A., Poland) [10].

3. Results

The costal cartilage samples collected for DNA analysis allowed us to obtain 7.636 ng/µL for T. Large Autosomal sequence, 8.888 ng/µL for T. Small Autosomal sequence, and 10.365 ng/µL for T. Y of high-quality DNA. The degradation index of DNA isolated from costal cartilage was 1.164. The concentrations of DNA isolated from personal belongings of the alleged victim are presented in Table 1. The highest concentration of DNA was obtained from a razor blade swab. Much lower concentrations were obtained successively from a toothbrush shaft swab, toothbrush filaments clippings, filter mask part adhering to the nose, filter mask elastic straps adhering to the earlobes. In all of the above locations, the DNA concentration was sufficient to obtain complete DNA profiles within the autosomal STR (Fig. 3) and Y STR loci (Fig. 4). Due to the very low con-

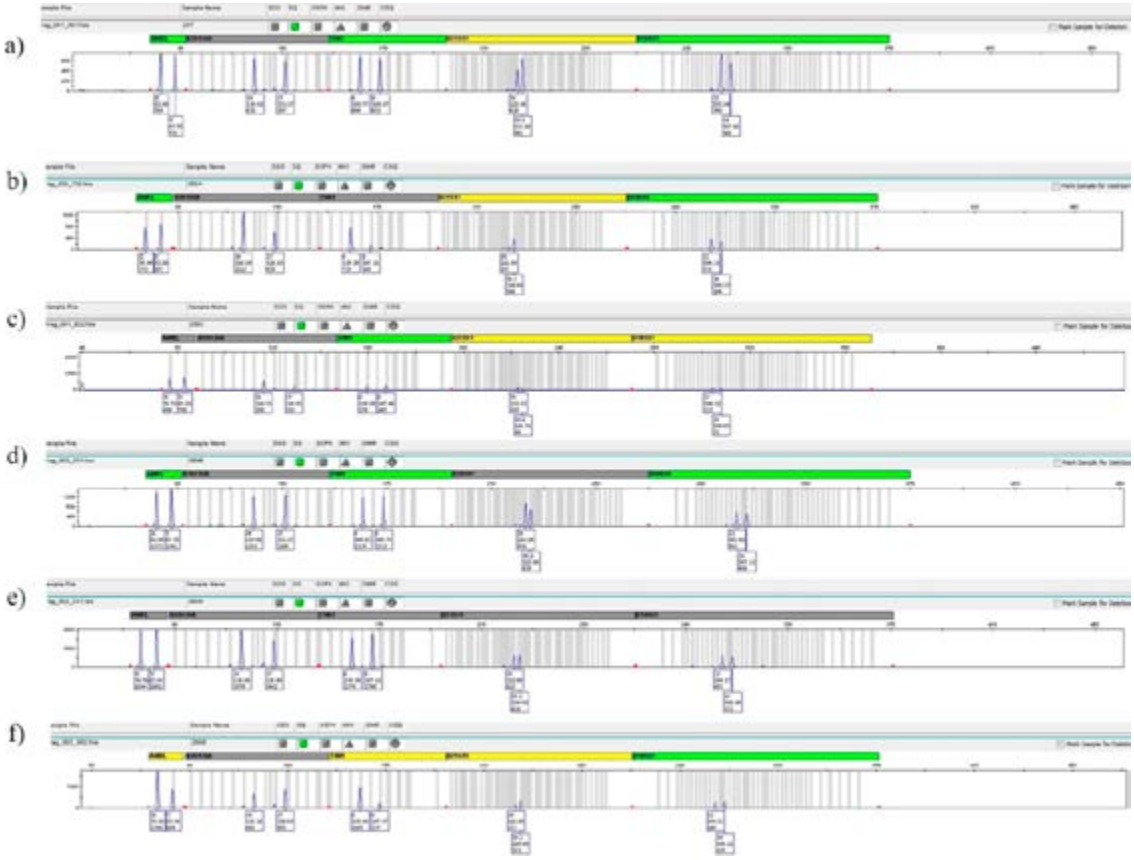

Fig. 3. DNA profiles (blue dye channel, PowerPlex® ESX 17 System) obtained from costal cartilage sample (a) and from personal belongings of the alleged victim: filter mask part adhering to the nose (b), filter mask elastic straps adhering to the earlobes (c), razor blade swab (d), toothbrush shaft swab (e), and toothbrush filaments clippings (f). The corresponding personal belongings are marked in Table 1.

centration of DNA obtained from the razor shaft swab and the central part of the filter mask, it was impossible to obtain DNA profiles for these samples. The degradation index for antemortem samples ranged from 1.164 to 2.872 and IPCCT flag was not triggered, which indicated that the isolated DNA was slightly degraded.

The results of DNA polymorphism analysis confirmed that the DNA profile of the immolated cadaver (DNA isolated from costal cartilage) is consistent with the DNA profile of the alleged victim (DNA isolated from personal belongings). The results showed that the evidence mentioned above evidence contained traces of DNA of the male cadaver under study. The probability of repeating the cadaver's genotype in the Polish population (calculated for 23 autosomal loci) was 1.52420×10^{-36}. The Y-STR haplotype (calculated for 16 loci) obtained from the male cadaver was observed twice among 4,957 haplotypes in the National Database – Poland, and three times among 277,004 haplotypes in the National Database – Worldwide, available at YHRD.org. On the other hand, the DNA profile compliance of the samples of the personal belongings with the DNA profile of the male cadaver is at least 6.48341×10^{32} times more likely, than if this match was accidental and the DNA would come from another, unrelated person. The likelihood ratio (LR) value from DNA testing provides solid evidence to support the hypothesis that the DNA from samples of personal belongings of the alleged victim is of male cadaver origin. The results of genetic tests allow assuming that the body of the male cadaver is the body of the alleged victim.

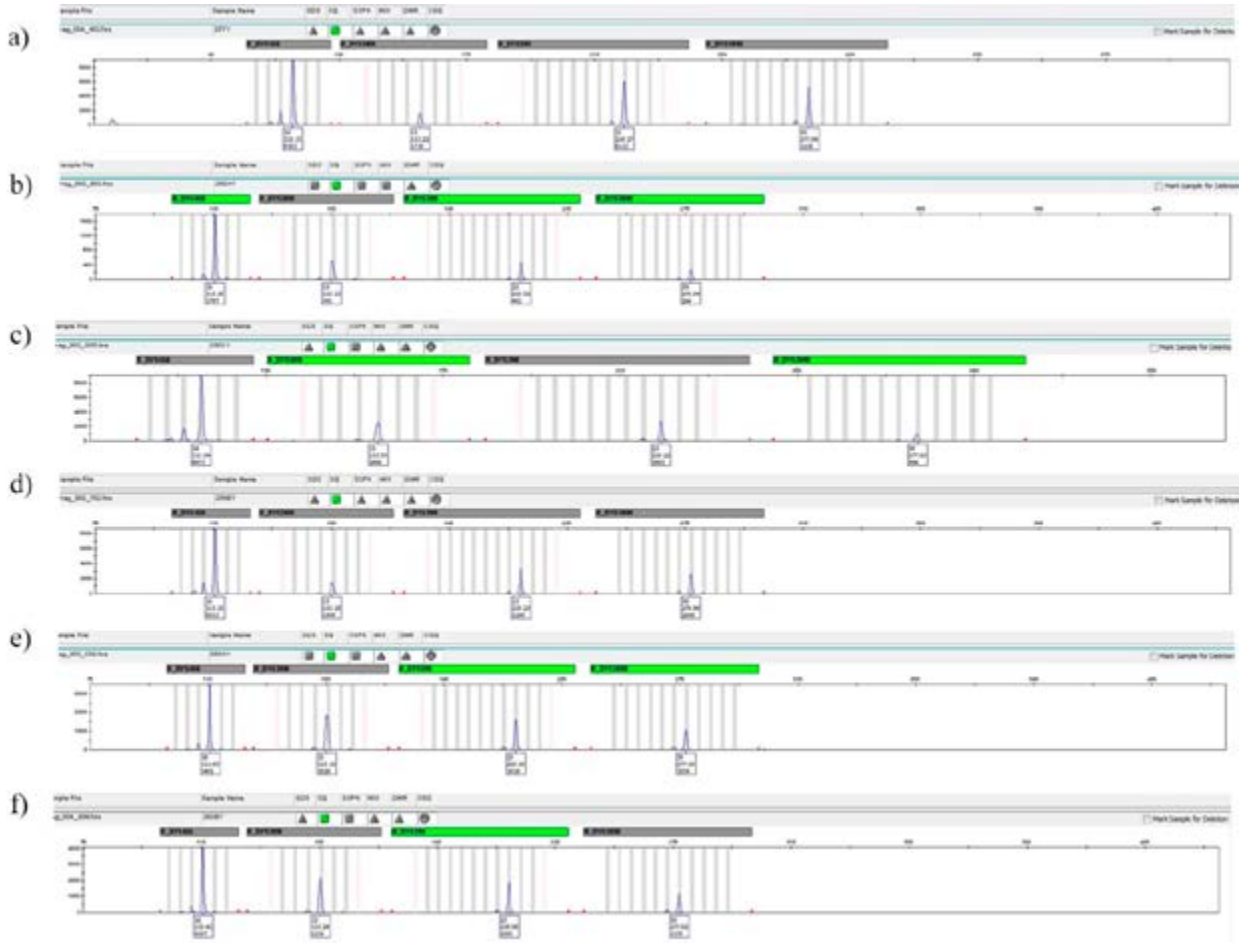

Fig. 4. DNA profiles (blue dye channel, AmpFlSTR® Yfiler™ System) obtained from costal cartilage sample (a) and from personal belongings of the alleged victim: filter mask part adhering to the nose (b), filter mask elastic straps adhering to the earlobes (c), razor blade swab (d), toothbrush shaft swab (e), and toothbrush filaments clippings (f). The corresponding personal belongings are marked in Table 1.

4. Discussion

Recently it was shown that it is possible to establish a genetic profile using CC samples [8]. CC can be helpful in cases with unknown blood transfusion status as this tissue is free from the chimerism phenomenon [9]. Apart from the search for alternative section materials for DNA profiling, alternative personal belongings are also wanted, and the optimization of DNA isolation methods from them is being researched. So far, filter masks have been underestimated as a forensic material and used only for research associated with the SARS-CoV-2 pandemic [12,13]. The case report presents the use of a disposable N95/FFP2 mask, which is the most frequently used respiratory protective device in healthcare globally [14,15]. We showed that it is possible to isolate human DNA from the filter mask clippings (except for its central part), the razor surface (except for the razor shaft), and the toothbrush. To isolate DNA from the toothbrush, we used a swab from its shaft and the 40 filaments clipped form its head. Two other studies on DNA isolation used the toothbrush head and its filaments. Tanaka et al. isolated higher DNA concentration from the whole toothbrush head incubated overnight and extracted using the

Table 1. DNA concentration and degradation index in samples collected from personal belongings of the alleged victim: 1 – filter mask part adhering to the nose; 2 – central part of the filter mask; 3 – filter mask elastic straps adhering to the earlobes; 4 – razor shaft swab; 5 – razor blade swab; 6 – toothbrush shaft swab; 7 – toothbrush filaments clippings

Sample	Target sequence	DNA quantity [ng/μL]	Degradation index
1	T. Large Autosomal	0.042	2.661
	T. Small Autosomal	0.112	
	T. Y	0.109	
2	T. Large Autosomal	0.001	1.250
	T. Small Autosomal	0.001	
	T. Y	0.002	
3	T. Large Autosomal	0.034	1.457
	T. Small Autosomal	0.049	
	T. Y	0.052	
4	T. Large Autosomal	0.002	2.871
	T. Small Autosomal	0.007	
	T. Y	0.008	
5	T. Large Autosomal	5.703	1.554
	T. Small Autosomal	8.863	
	T. Y	8.591	
6	T. Large Autosomal	0.083	1.879
	T. Small Autosomal	0.156	
	T.Y	0.184	
7	T. Large Autosomal	0.060	2.149
	T. Small Autosomal	0.128	
	T. Y	0.104	

phenol-chloroform method [16]. Riemer et al., using the distal part of the toothbrush head, also isolated higher DNA concentration than reported in our case report. However, in 7.3% of cases, they could not obtain the complete DNA profile [17].

Another important aspect of using personal belongings in DNA profiling is the time that has passed since the last use of the items. According to the Prosecutor's Office, in the described case, it could have been several days. However, Riemer et al., who determined DNA concentration in the toothbrush one and three months after use, found no statistically significant differences in the quantity of isolated DNA [17]. Thus, the four-day postmortem interval (PMI) in the presented case and the several-day period that had passed since the last use of personal belongings probably did not significantly reduce the amount of DNA available for isolation.

According to our knowledge, the filter masks have not been analyzed in cases of personal identification yet. We recommend further studies on the filter masks applications in forensic genetic analysis.

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