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Recommendations for the successful identification of altered human remains using standard and emerging technologies: results of a systematic approach

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Conflict of interests

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Abstract

Successful DNA-based identification of altered human remains relies on the condition of the corpses and varies between tissue types. Therefore, the aim of this prospective multicenter study was to generate evidence-based recommendations for the successful identification of altered remains. For this, 19 commonly used soft and hard tissues from 102 altered human bodies were investigated. The corpses' condition was categorized into three anatomical regions using a practical scoring system. Besides other data, DNA yields, degradation indices, and short tandem repeat (STR) profile completeness were determined in 949 tissue samples. Additionally, varying degrees of alteration and tissue-specific differences were evaluated using the Next Generation Sequencing (NGS) platform MiSeq FGx™. Selected challenging samples were sequenced in parallel with the Ion S5™ platform to

assess platform-specific performances in the prediction of the deceased's phenotype and the biogeographic ancestry.

Differences between tissue types and DNA extraction methods were found, revealing, for example, the lowest degradation for vertebral disc samples from corpses with initiating, advanced and high degrees of decomposition. With respect to STR profile completeness, blood samples outperformed all other tissues including even profoundly degraded corpses. NGS results revealed higher profile completeness compared to standard capillary electrophoresis (CE) genotyping. Per sample, material and degradation degree, a probability for its genotyping success, including the "extended" European Standard Set (eESS) loci, was provided for the forensic community. Based on the observations, recommendations for the alteration-specific optimal tissue types were made to improve the first-attempt identification success of altered human remains for forensic casework.

Keywords

Human identification, corpse classification, decomposition, MiSeq FGx™, Ion S5™, recommendations

Introduction

Short tandem repeat (STR) genotyping is essential for identifying altered human remains including decomposed, burnt corpses and bodies found in water, and is particularly important when no reference data like computed tomography scans or fingerprints are available [1]. Yet, the DNA-based identification (ID) success relies on the quantity and quality of the extracted DNA [2]. Extended post-mortem intervals (PMI) with associated decomposition processes and exposure to high temperatures, for example, can reduce DNA integrity and overall amplification success [2-5]. Also, proceeding microbial growth augments DNA degradation [6], and polymerase chain reaction (PCR) inhibitors like humic compounds, produced during decay processes, or Ca^{2+} ions, from dry bones, can impair the polymerase activity during PCR [7, 8]. Since the degree of decomposition depends on several factors like environmental conditions, which can impact the human body unevenly, the extent of DNA degradation can strongly vary among tissue types [2, 9-12]. Therefore, the question of the right sampling material arises which directly affects STR genotyping and the desired ID success of altered human remains [5, 10, 11, 13].

To our knowledge, a systematic approach and recommendations for identification do not exist for a large variety of soft and hard tissues from decomposed, burnt, and submerged corpses with varying degrees of alteration. A comparison of previous studies revealed mostly small sample sizes and partly contradicting recommendations [14]. In the field of disaster victim identification, for example, recommendations and strategies exist that provide valuable guidance for sample collection and

prioritize bone samples of decomposed corpses [15, 16]. Accordingly, long, compact bones, healthy teeth, and/or other available bones should be the first choice for decomposed corpses. However, bone samples can be sensibly circumvented if soft tissues are still available as osseous preparation is more time-consuming, tedious and requires well-trained staff [9, 17, 18]. Therefore, multiple studies describe alternative sample materials like bladder swabs [3, 12], nails [11, 19, 20] or soft tissues like intervertebral discs [4], organs [9, 17] or the Achilles tendon [10]. Those diverging recommendations and observations from a previous study [14] highlight an uncertainty in the choice of the best-suited tissue according to its degree of decomposition. Furthermore, the corpse's condition is usually not or not optimally scored due to challenges in categorizing the alteration processes even within one body. Thus, varying DNA laboratory-specific processes and a missing classification system render published study results less comparable and impede their reproducibility.

Most studies focus on capillary electrophoresis (CE) analysis, which is considered the gold standard in forensic genetics [21-25]. However, the proceeding development of high throughput DNA sequencing technologies leads to their growing relevance in forensic casework and provides promising approaches for the analysis of altered remains by expanding the spectrum of forensic DNA investigations [22, 24, 26-28]. Next Generation Sequencing (NGS) methods allow multiplexing autosomal and gonosomal STRs, as well as SNPs on a much larger scale. The reduction of amplicon length is of benefit for degraded samples and displays an additional advantage compared to CE [26, 27, 29]. As a previous study has shown, NGS genotyping revealed significantly lower numbers of allelic dropouts compared to CE when analyzing autosomal STR profiles from artificially degraded blood samples [29]. Furthermore, the potential to predict a deceased's phenotype and biogeographic ancestry within a given legal framework can add valuable information about the person's identity and assist investigative leads [30, 31].

Using a systematic approach, this multicenter study aimed to establish recommendations on the optimal tissue types for a DNA-based ID of altered human remains according to the respective degree of alteration at first attempt. By evaluating a broad variety of different soft and hard tissues and comparing DNA extraction methods of the Institutes of Forensic Medicine nameX and nameY, corpse material with the highest probability of STR genotyping success was determined. Besides standard CE analysis, NGS was completed for suitable samples to explore potentially better performance on degraded and inhibited samples [27, 29]. Next, using the leading site's MiSeq FGx^{TM} technology and the Ion S5^{TM} system of the Institute of Forensic Medicine nameZ, selected challenging samples were sequenced to assess platform-specific prediction power on the phenotype and the biogeographic ancestry of the deceased. Finally, recommendations were represented that are fast and easy to implement in routine forensic casework to standardize the choice of the optimal tissue type for an improved first-attempt identification success of altered human remains.

Material and methods

Sample collection

Over a period of three years, 949 samples from soft tissues (about 500 mg of heart, lung, spleen, kidney, liver, *M. rectus femoris*, *M. pectoralis major*, aorta), hard tissues (rib, *pars petrosa*, vertebra, femur, humerus, whole toenail, whole fingernail) and body fluids (blood, buccal swabs, bladder swabs) were collected from human bodies during medico-legal autopsies or identifications at the Institutes of Forensic Medicine nameX and nameY. Decomposed corpses (n = 91 nameX, n=4 nameY), bodies found in water (n = 5, nameX) and burnt bodies (n = 2, nameX) showed varying signs of decomposition or burning (Tab.1) and post-mortem intervals (PMI) from < 24 hours to several years. Tissue samples from unaltered corpses (n = 5, nameX) were collected as the control group, leading to a total of 107 human bodies. The study design and sampling were approved by the regional Ethical Review Board (No. 2019-02211).

Scoring method for measuring the degree of decomposition and burning

For grading the extent of decomposition and burning, human remains were categorized into five categories according to the body's condition ranging from no (D_0) to severe degradation (D_5), prior to the medico-legal autopsy or identification (Tab.1). Due to possibly deviating states of alteration throughout the body, the parameters were scored independently for three anatomical regions: 1) the head (including the neck), 2) the trunk (thorax, abdomen and pelvis), and 3) the limbs (arms and legs). The stages of decomposition were classified using partially modified methods and categorizations [32-37] as outlined in Tab. 1. The three anatomical regions' scores were not summed to a Total Body Score (TBS) [32] because some of the examined bodies with different decomposition patterns per region resulted in the same TBS.

Sample preparation, DNA extraction, quantification, amplification and capillary electrophoresis

Osseous samples were processed with a modified protocol adapted from Pajnic [38]. Subsequent to removing the remaining tissue with scalpels, the bones were manually cleaned with distilled water (Qiagen, Venlo, NL), 5% Alconox (Alconox, Inc., White Plains, NY) and ethanol to eliminate adherent contaminants. The bone surface was polished with a sanding tool (Dremel, Racine, WI) under a fume hood (Erlab, Rowley, MA) and then dried at 50°C for 2h in an incubator (Labnet International, Edison, NJ). In case the bone was too wet, it was dried at 50°C for 2h prior to surface polishing. To ensure the removal of contaminants, the washing and drying steps were repeated. Following the fragmentation of the bone in a DNA-free bag using a hammer, the shattered pieces were pulverized using a tube mill (Tracomme, Schlieren) and 100 mg of the gained bone powder was used for DNA extraction with the Bone DNA Extraction Kit (Promega, Madison, WI). Genomic DNA was extracted

from blood (15 µl), soft tissue samples (100 mg each), nails (1 mm^2 from the nail bed) and swabs (ThermoFisher Scientific, Waltham, MA) using two extraction methods eluted in 50 µl each: 1) the Maxwell® FSC DNA IQ™ Casework Kit (MWK, Promega) on the Maxwell RSC instrument (Promega) and 2) the SwabSolution™ Kit (SSK, Promega) according to the manufacturer's protocols [39, 40].

Tissue samples from the decomposed corpses collected at the Institute of Forensic Medicine nameY were extracted with the institute-specific extraction method to determine the influence of DNA extraction methods. For soft tissues, the iPrep™ Forensic Kit (IPK, ThermoFisher Scientific) was used. For bone samples, the PrepFiler Express BTA™ Kit (BTA, ThermoFisher Scientific) was used as described in [41] with the following modifications: in the cell lysis step, the volume for PrepFiler Express BTA™ was doubled, directly added to 100 mg bone sample and incubated overnight at 56°C. Both kits were not separately analyzed and are thus referred to as IPK/BTA method.

DNA quantification of all 1698 extractions was performed on the 7500 Real-Time PCR System (Applied Biosystems, Waltham, MA) in a total reaction volume of 25 µl using the Plexor HY System (Promega) according to the manufacturer's protocols [42, 43]. For samples extracted with the SSK method, the recommended 5X AmpSolution™ Reagent (Promega) was added, as the absence of the reagent can inhibit subsequent analyses [40]. Subsequent to amplifying 22 STR loci with the Investigator 24Plex QS Kit (further referred to as 24Plex, Qiagen), a fragment length analysis was performed on the ABI 3500 xL Genetic Analyzer (Applied Biosystems). The 24Plex kit includes the European Standard Set (ESS): FGA, TH01, VWA, D1S1656, D2S441, D3S1358, D8S1179, D10S1248, D12S391, D18S51, D21S11, D22S1045, the additional loci D2S1338, D16S539, D19S433, SE33 (further referred to as *extended ESS (eESS) loci*), as well as the STR loci TPOX, DYS391, CSF1PO, D5S818, D7S820, D13S317 plus Amelogenin and two quality sensors [44-46]. All analyses included the required positive and negative controls.

Table 1: Categorisation of the corpses' degrees of alteration in three anatomical regions. Decomposed corpses were classified according to Megyesi et al. [32] and Gelderman et al. [33] and bodies found in water according to van Daalen [34], Heaton [35] and Reh [36]. Degrees of burning were classified according to the Crow-Glassman scale [37], Dettmeyer et al. [1] and Symes et. al. [47]

Condition	Degree	Classification	Description		
			Head	Trunk	Limbs
Decomposed	D_0	Unaltered	No visible alteration	No visible alteration	No visible alteration
	D_1	Initiating	Livor mortis, rigor mortis, drying of nose, lips and ears	Livor mortis, rigor mortis, skin appears pink-white	Livor mortis, rigor mortis, drying of finger and toes
	D_2	Advanced	Bloating of the face, formation of putrefactive blisters, grey to green discoloration, skin slippage	Resounding blood vessels, formation of putrefactive blisters, grey to green discoloration, skin slippage	Resounding blood vessels, formation of putrefactive blisters, grey to green discoloration, skin slippage

	D_3	Highly	Extensive green to black discoloration, moist and detachable skin, purging of putrefaction fluids	Abdominal bloating, extensive green to black discoloration, moist and detachable skin, purging of putrefaction fluids	Extensive green to black discoloration, moist and detachable skin, purging of putrefaction fluids
	D_4	Profoundly	Partial skeletonization, loss of organic/inorganic substances, caving in of the flesh and tissue of eyes	Partial skeletonization, loss of organic/inorganic substances, caving in on the abdominal cavity	Partial skeletonization, loss of organic/inorganic substances, joints still articulated
	D_5	Skeletonized	Complete skeletonization	Complete skeletonization	Complete skeletonization
Found in water	D_0	Unaltered	No visible alteration	No visible alteration	No visible alteration
	D_1	Initiating	Livor mortis, rigor mortis, darkened lips, slight pink discoloration	Livor mortis, rigor mortis, slight pink discoloration	Livor mortis, rigor mortis, wrinkling of skin on hands and feet
	D_2	Advanced	Bloating of the face, formation of putrefactive blisters, grey to green discoloration, skin slippage	Resounding blood vessels, formation of putrefactive blisters, grey to green discoloration, skin slippage	Resounding blood vessels, formation of putrefactive blisters, grey to green discoloration, degloving and/or absence of nails
	D_3	Highly	Extensive green to black discoloration, moist and detachable skin, purging of putrefaction fluids	Abdominal bloating, extensive green to black discoloration, moist and detachable skin, purging of putrefaction fluids	Extensive green to black discoloration, moist and detachable skin, purging of putrefaction fluids
	D_4	Profoundly	Partial skeletonization, loss of organic/inorganic substances, caving in of the flesh and tissue of eyes	Partial skeletonization, loss of organic/inorganic substances, caving in on the abdominal cavity	Partial skeletonization, loss of organic/inorganic substances, joints still articulated
	D_5	Skeletonized	Complete skeletonization	Complete skeletonization	Complete skeletonization
Burnt	D_0	Unaltered	No visible alteration	No visible alteration	No visible alteration
	D_1	Level 1	Blistering of upper dermal layer, red skin	Blistering of upper dermal layer, red skin	Blistering of upper dermal layer, red skin
	D_2	Level 2	Damage of dermis, coagulation necrosis	Damage of dermis, coagulation necrosis	Damage of dermis, coagulation necrosis, pugilistic posture
	D_3	Level 3	Dermis/subcutaneous fat tissue completely burnt	Dermis/subcutaneous fat tissue completely burnt	Dermis/subcutaneous fat tissue completely burnt, parts of arms and/or legs missing
	D_4	Level 4	Extensive burn destruction, heat-induced bursting of the cranium	Extensive burn destruction	Extensive burn destruction
	D_5	Level 5	Cremation with little or no tissue left	Cremation with little or no tissue left	Cremation with little or no tissue left

Next Generation Sequencing with the MiSeq FGx™ and the Ion S5™ system

A total of 155 randomly selected tissue samples, with all degrees of decomposition represented, were sequenced using the ForenSeq™ DNA Signature Prep Kit on the MiSeq FGx™ System (Verogen, San Diego, CA). Target amplification was performed with DNA Primer Mix A (DPMA: 27 autosomal STRs, 24 Y-STRs, 7 X-STRs, 94 identity informative (ii) SNPs) and DNA Primer Mix B (DPMB: 22 phenotypic informative (pi) SNPs, 56 biogeographical ancestry informative (ai) SNPs and the DPMA

loci) in reaction volumes of 15 µl. Target enrichment, library purification, normalisation, pooling, and denaturation of libraries were conducted according to the manufacturer's protocol [48]. To assure the libraries' quality prior to sequencing on the MiSeq FGx™ micro flow cells (Verogen), the High Sensitivity DNA Kit on the Bioanalyzer 2100 (Agilent, Santa Clara, CA) was used as quality control. Each sequencing run included 2800 M Control DNA (Promega) as a positive control and nuclease-free water (Qiagen) as a negative control.

For possible device-dependent deviations in predicting the deceased's phenotype and biogeographic ancestry, a subset of 20 tissue types D_2 to D_4 were additionally sequenced using the Ion S5™ (ThermoFisher Scientific) at the Institute of Forensic Medicine nameZ. For this, the leading site provided quantified DNA extracts from the heart, Achilles tendon, aorta, vertebral disc, *M. rectus femoris*, lung, teeth, rib, blood samples, toenails and bladder swabs. For each sample, the Precision ID Ancestry Panel (Applied Biosystems) and HIrisPlex-S Panel (AmpliSeq Designer Panel) [49] were analysed together. The library preparation was performed on the Ion Chef™ (ThermoFisher Scientific) using the Precision ID DL8 Kit (Applied Biosystems). With exception of the mixture of both primer panels, which is described in the ThermoFisher Technical Note [50], the library preparation was performed according to the manufacturer's protocols [51, 52]. Sequencing was performed on the Ion S5™ using the Ion S5™ Precision ID Chef & Sequencing Kit (Applied Biosystems) according to the manufacturer's protocol on an Ion 520™ Chip (Ion Torrent™) [51].

Data analysis

Quantification was performed using the Plexor Analysis Software (Promega, version 1.5.6.7) according to the manufacturer's instructions [42]. CE data were analysed by using the GeneMapper ID-X v.1.6 Software (Applied Biosystems) with default stutter filters and a validated analytical threshold of 50 relative fluorescence units (RFU). For each corpse, a reference STR profile was generated by combining reportable alleles derived from all of the corpse's analysed tissue samples (composite profile) or by using previous STR information from the respective case. Profile completeness was calculated separately for 1) successful typing of all kit included 22 STR loci and 2) successful typing of the 16 eESS loci. Peak heights below the analytical threshold were interpreted as dropouts. Profile completeness in percentage was calculated by dividing the reportable alleles by the number of alleles from the corpse's reference profile. Additionally, the probability of genotyping success was calculated separately for 22 and 16 loci (further referred to as probability of genotyping success) by dividing the number of complete profiles by the number of samples.

For average peak height, all allele heights were summed and divided by the number of alleles. The 24Plex kit-specific quality sensors QS1 (74 bp) and QS2 (435 bp) were used to assess the presence of PCR inhibitors and confirm DNA degradation [53]. The ratio of the sensors, calculated by dividing the peak height of QS1 by the peak height of QS2, indicates inhibited DNA in case of decreasing peak

heights for QS2 [53]. Besides the interpretation of the artificial quality sensors, an additional degradation index (DI) was calculated to assess the true extent of DNA degradation by using the genetic material itself with the following equation:

$$DI = \left(\frac{peak\ height\ D21S11}{peak\ height\ THO1} + \frac{peak\ height\ SE33}{peak\ height\ TPOX} + \frac{peak\ height\ D2S1338}{peak\ height\ D10S1248} + \frac{peak\ height\ FGA}{peak\ height\ D2S441} + \frac{peak\ height\ D7S820}{peak\ height\ D16S539} \right) / 5$$

The resulting DI ranged between 1 (no degradation) and 0 (complete degradation) [54].

MiSeq FGx™ sequencing data were analysed using the ForenSeq Universal Analysis Software (UAS, Verogen) with default interpretation and analytical threshold settings. Coverage below the analytical threshold of 1.5% was considered as allelic dropout. For each corpse, the reference profile generated by the CE-based STR (CE STR) was used. Completeness between CE and NGS profiles was compared using the overlapping STR loci Amelogenin, TPOX, FGA, TH01, VWA, D1S1656, D2S1338, D2S441, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, DYS391, D22S1045 and CSF1PO. The polymorphic STR locus SE33 (ACTBP2) was not included [55]. Estimation of biogeographic ancestry and prediction of phenotype was provided by the UAS. Sequencing data by the Ion S5™ were analysed using the Converge™ Software (ThermoFisher Scientific) with default interpretation and analytical threshold settings. DNA phenotyping was performed using the HIrisPlex-S online prediction tool (https://hirisplex.erasmusmc.nl/) [56-58] and estimation of biogeographic ancestry was obtained from Converge™ Software. For the comparison of the platform-specific predictions on phenotype and ancestry, a NGS profile was defined as complete when all loci were reportable per MiSeq FGx™ (24 piSNPs, 54 aiSNPs) and Ion S5™ kit (42 piSNPs, 165 aiSNPs), respectively.

Statistical analyses were performed using R version 4.1.1 [59] and R studio version 2021.09.0 [60]. The distribution of the data was assessed with the Shapiro-Wilk normality test, density and Q-Q plots using the *dplyr* [61] and *ggpubr* [62] packages. Normality could be assumed for DNA quantity and RFU peak heights. Then, one-, two- and three-way factor analyses of variances (ANOVA) were used to determine statistical significance for the influence variables *degrees of alteration*, *tissue types* and *DNA extraction method* (with and without interaction). For this, the package *lpsolve* [63] as well as the function *aov* and *TukeyHD* were used and significance was defined as $p<0.05$. Since no normal distribution could be assumed for DI, QS and profile completeness (in percent), the nonparametric Kruskal-Wallis test was used in these cases to determine significant differences for either *degree of decomposition*, *tissue type* or the *extraction methods* MWK and SSK. The attainment of a complete STR profile is a dichotomous outcome and was thus analysed by a logistic regression with influence variables *tissue type*, *degree of decomposition* and *extraction method*. Comparison of profile completeness between NGS and CE as well as profile completeness for the piSNPs and aiSNPs for phenotype and ancestry prediction between the MiSeq FGx™ and Ion S5™ systems was assessed by

using the paired Wilcoxon signed-rank test. Data visualisation was performed using the *ggplot2* package [64].

Results

DNA quantification

DNA quantities of samples from decomposed corpses differed significantly between *tissue types* ($p<0.001$) and *degrees of decomposition* ($p<0.001$) but not for the *extraction method* ($p=0.632$). Significant interactions between *extraction method* and *degree* ($p<0.001$) and between *extraction method* and *tissue type* ($p<0.001$) were found (three-way ANOVA). Stratified analyses for the degree of decomposition showed a significant influence in DNA *extraction method*, but only in unaltered human remains (D_0, $p<0.001$), and a significant influence on *tissue type* for degrees D_0-D_3 (all $p<0.001$). Interactions were significant for D_1, D_2 and D_3 (two-way ANOVA).

For DNA extracted with the MWK method, yields ranged from 3 pg/µl to 748 ng/µl and showed the highest mean concentrations in kidney and spleen samples from unaltered remains (222 ng/µl, and 211 ng/µl, respectively) (Fig. 1A). As expected, for most tissue types, the DNA yield decreased with advanced decomposition processes. The lowest DNA yields were measured for teeth samples in each degree of decomposition. Wide confidence intervals indicated high variations even within the same tissue type and degree of decomposition and showed the greatest range for liver samples D_1 (3 pg/µl to 314 ng/µl). In contrast, confidence intervals of samples from the vertebral disc, aorta and blood were smaller and more consistent between degrees of decomposition, indicating low variances. DNA extracted with the SSK method displayed a higher concentration range from 4 pg/µl to 1374 ng/µl. Spleen sample D_1 yielded the highest mean DNA concentrations (530 ng/µl) and samples from the Achilles tendon the lowest within each degree of decomposition (Fig. 1B). For comparing the efficiency of DNA extraction methods, 57 D_3 or D_4 tissue samples were extracted with the IPK/BTA method, which showed highest mean DNA yields for spleen samples D_4 and fingernails D_3 (158 ng/µl, 104 ng/µl, respectively). With the small sample size (n=4), no samples D_0, D_1, D_2 and D_5 could be collected at the Institute of Forensic Medicine nameY.

For bodies found in water (n=5), only tissue samples D_0, D_1 and D_3 could be obtained. As for decomposed bodies, DNA yields decreased with advanced signs of decomposition. The highest yields were observed for spleen samples D_1 with mean amounts of 692 ng/µl, while buccal swabs D_3 resulted in the lowest yields with 0.83 ng/µl, both extracted with the SSK method. DNA yields of burnt human remains (n=2) ranged from 4 pg/µl to 514 ng/µl and showed the lowest yields of DNA from the *M. rectus femoris* D_3 (5 pg/µl MWK) and highest from spleen samples D_3 (166 ng/µl MWK).

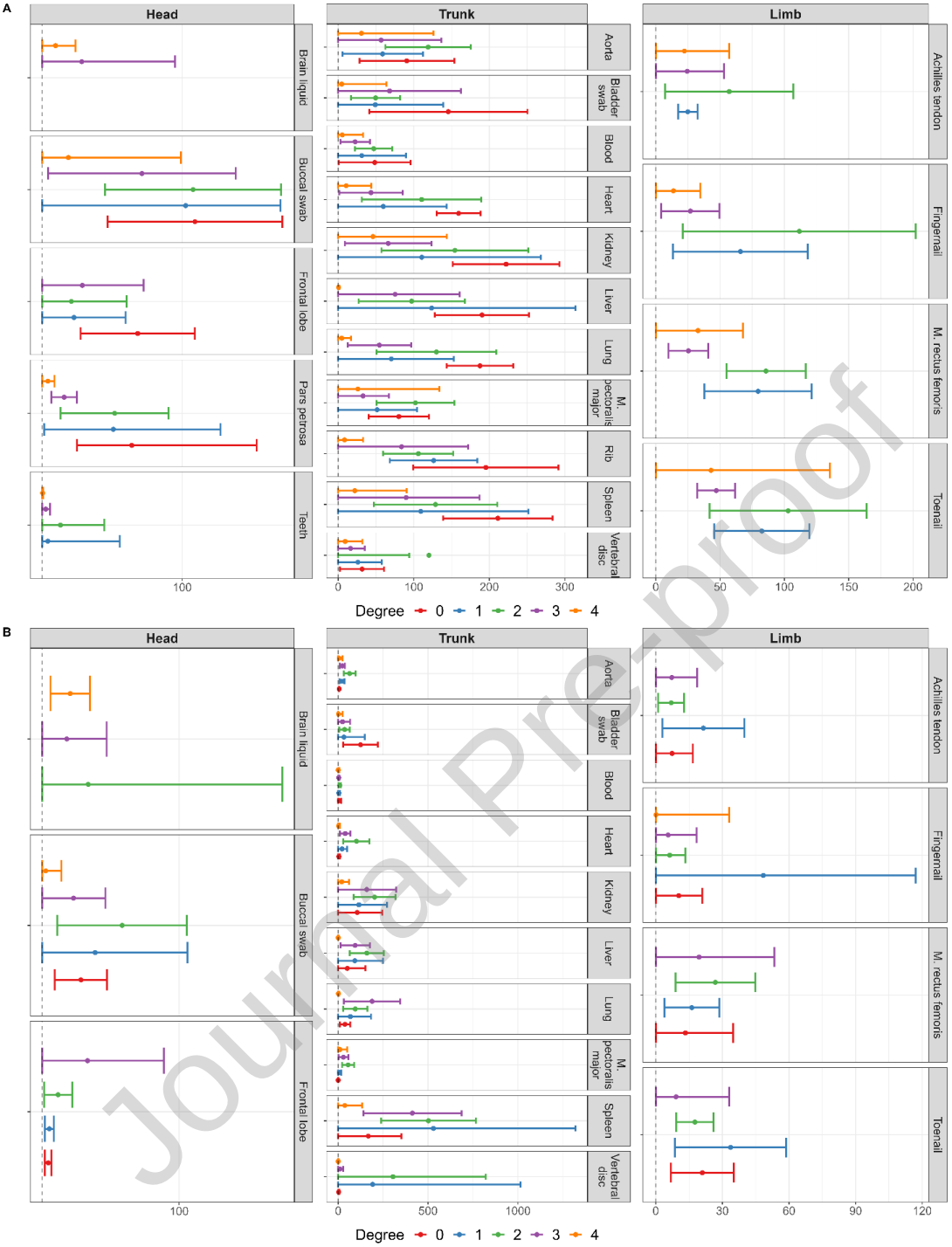

Figure 1: Quantification results (ng/µl) of DNA extracted with the Maxwell® FSC DNA IQ™ Casework Kit (A) and SwabSolution™ Kit (B) from tissue samples of decomposed corpses. Presented is the confidence interval of the mean. Samples are separated according to the anatomical regions and the corpse's degrees of decomposition ranging from 0 (unaltered) to 4 (profoundly). Since the SwabSolution™ Kit is not suitable for bone samples, D_5 results are not available. Due to the small number of samples, D_5 results are not shown for Maxwell® FSC DNA IQ™ Casework Kit. The other missing data represent unavailable sample material.

DNA integrity

For decomposed corpses, the calculated degradation indices differed prominently by *tissue types* ($p<0.001$ MWK, $p<0.001$ SSK), *degrees of decomposition* ($p<0.001$ MWK, $p<0.001$ SSK) and extraction method ($p<0.001$). Stratified analyses showed significant differences in *extraction method*

for D_2 (p=0.001), D_3 (p<0.001) and D_4 (p<0.001) and *tissue type* for D_0-D_3 (p<0.001, all p-values Kruskal-Wallis test). For DNA extracted with the MWK method, low DI of bladder swabs D_4 (median = 0.19), buccal swabs D_4 (median = 0.07), kidney samples D_4 (median = 0.07), and *pars petrosa* samples D_5 (median = 0.01) indicated a strong "ski-slope effect" [65] and therefore highly degraded DNA (Fig. 2A). With the exception of D_4, vertebral disc samples revealed the highest DI of > 0.80 for each degree of decomposition and, thus, low DNA degradation. For the SSK method, samples from the liver and fingernail D_4 revealed the lowest DI (Fig. 2B). Indices of kidney and liver samples were < 0.15 for each degree of decomposition, representing highly degraded DNA. For tissue samples extracted with the IPK/BTA method, the DI was comparably low for each analyzed tissue type. The highest degradation was observed for Achilles tendon D_4 (DI median = 0), and the lowest for samples from blood D_4 (DI median = 0.93) and ribs D_3 (DI median = 0.78).

Bodies found in water also showed lower DI with advanced signs of putrefaction. For D_3, DNA from liver samples and buccal swabs displayed the lowest median DI for both the MWK and SSK extraction methods (0.09, and 0.11, respectively). DNA from burnt corpses was most degraded in muscle samples (*M. rectus femoris* and *M. pectoralis major*) and showed DI of > 0.001 even for D_1. In contrast, buccal swabs D_3 showed the highest DI median indices (0.98) with the MWK method.

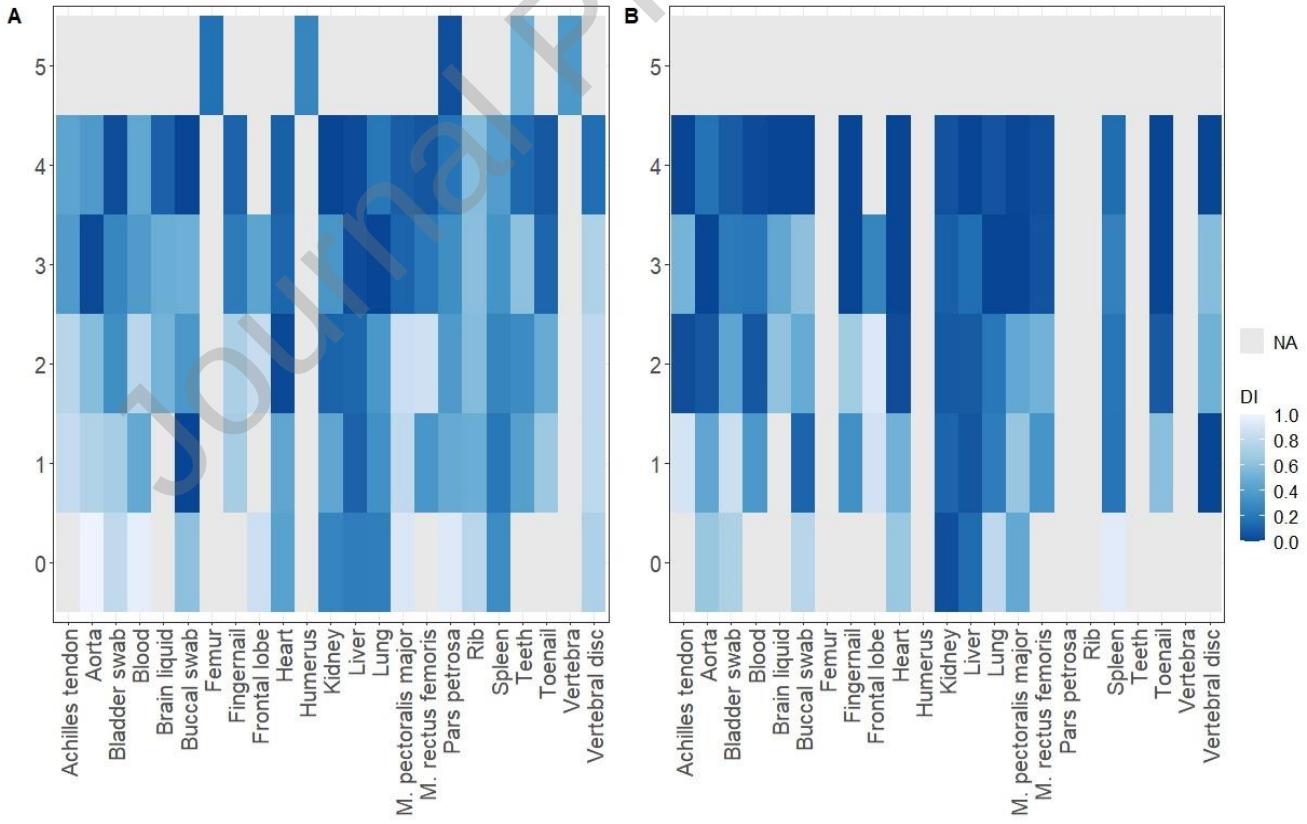

Figure 2: Heatmap of degradation indices (DI) of DNA extracted with the Maxwell® FSC DNA IQ™ Casework Kit (A) and the SwabSolution™ Kit (B). Tissue samples were collected from putrefied corpses classified in degrees of decomposition ranging from 0 (unaltered) to 5 (skeletonized). Missing data represents unavailable sample material.

The ratio of peak heights of internal quality sensors ranged from 0 to 2.13 (MWK) and 0 to 1.59 (SSK) for decomposed corpses (Fig. S1). DNA extracted with the MWK method showed no significant differences between *tissue types* (p=0.968) but between *degrees of decomposition* (p<0.001, all p-values Kruskal-Wallis test). For D_0, a median of 0.95 indicated balanced peak heights and no presence of inhibitors. With advanced degree of decomposition, the median only slightly increased from 1.03 (D_1) to 1.09 (D_5), which reflects absence of inhibitors and an efficient purification of the DNA extracts. For three samples the larger quality sensor QS2 dropped out, thus implying the presence of inhibitors. For samples extracted with the SSK method, the ratios differed significantly between *tissue types* (p=0.011) and *degrees of decomposition* (p<0.001, all p-values Kruskal-Wallis test). With a median of 0.91 (D_0) and 0.85 (D_4), a decrease in QS2 peak heights was observed, indicating advanced degrees of decomposition. Compared to the MWK method, more samples were below 0. In comparison, DNA extracted with the IPK/BTA method exposed less ratios below 0, with a median of 1.12 (D_3) and 1.37 (D_4).

For bodies found in water, results were comparable to decomposed bodies and showed medians of 1.01 (D_1) to 1.11 (D_3) for samples extracted with MWK and 0.94 (D_1) to 0.87 (D_3) for SSK. Medians of burnt human remains ranged from 1.09 (D_1) to 1.21 (D_3) for samples extracted with MWK and 1.34 (D_1) to 1.0 (D_3) for samples extracted with SSK.

STR genotyping with CE (CE STR)

RFU peak heights

RFU peak heights of samples from decomposed corpses differed significantly between *tissue types* (p<0.001), *degrees of decomposition* (p<0.001) and *extraction method* (p<0.001). Significant interactions between the extraction method and degree (p<0.001) were found (three-way ANOVA). Stratified analyses for the degree of decomposition showed a significant influence in DNA *extraction method* only in D_2 (p<0.001) and D_0 (p<0.001) and significant influence in *tissue type* for degrees D_1-D_4 (all p<0.001). Interactions were significant for D_2 (two-way ANOVA).

The highest mean peak heights were noted in MWK extracted samples from the frontal lobe D_1 and D_3 (7279 RFU, 6497 RFU, respectively) as well as heart D_3 (4061 RFU) (Tab. S1). In comparison, the mean peak heights of liver samples were lower for each degree of decomposition. For SSK extracts, greater variations in each degree of decomposition were observed, with the highest mean heights for heart samples D_1 (3002 RFU) and the lowest for lung samples D_4 (540 RFU). For tissue IPK/BTA extracted samples, no great differences were shown for samples D_3 and D_4. Mean heights of blood samples D_4 (6457 RFU) and samples from the frontal lobe D_3 (4562 RFU) were the largest and *pars petrosa* samples D_4 revealed the lowest peak heights (875 RFU).

Mean peak heights of samples from bodies found in water also varied between the *degree of decomposition* as well as *tissue types* and ranged from 564 to 9695 RFU (MWK) and 55 to 26042 RFU (SSK). Comparable to samples from decomposed remains, DNA extracted with the SSK method revealed a greater variation within each degree and the greatest mean heights for samples from the *M. pectoralis major* D_3 (6416 RFU SSK) and the frontal lobe D_1 (5312 RFU MWK). The low number of samples from burnt human remains showed high peak height variations within both extraction methods and the greatest heights in samples from the vertebral disc D_3 (8406 RFU MWK) and fingernails D_3 (14820 RFU SSK) as well as lowest in samples from the liver D_3 (1996 RFU MWK) and *M. rectus femoris* D_3 (109 RFU SSK).

Profile completeness

Statistical differences in profile completeness of the 22 STRs were observed for *tissue types* ($p<0.001$ MWK, $p<0.001$ SSK) as well as the *degree of decomposition* ($p<0.001$ MWK, $p<0.001$ SSK) (Fig. 3). Although the DNA extraction methods differed statistically ($p<0.001$, all p-values Kruskal-Wallis test), differences were not significant in the subgroups D_0, D_1 and D_2. As expected, profile completeness decreased with advanced signs of decomposition. Median profile completeness of MWK extracted heart samples revealed a constant decline from 99% (D_0), 99% (D_1), 98% (D_2), 91% (D_3) to 86% (D_4) (Fig. 3A). However, DNA from blood samples showed no decrease and median profile completeness of 100% for each degree. As a comparison, the profile completeness of each SSK extracted tissue displayed significantly more allele dropouts for D_3 and D_4 (Fig. 3B). Due to greater variances within each tissue and degree, median profile completeness of heart and vertebral disc samples D_4 decreased to 29% and 28%, respectively.

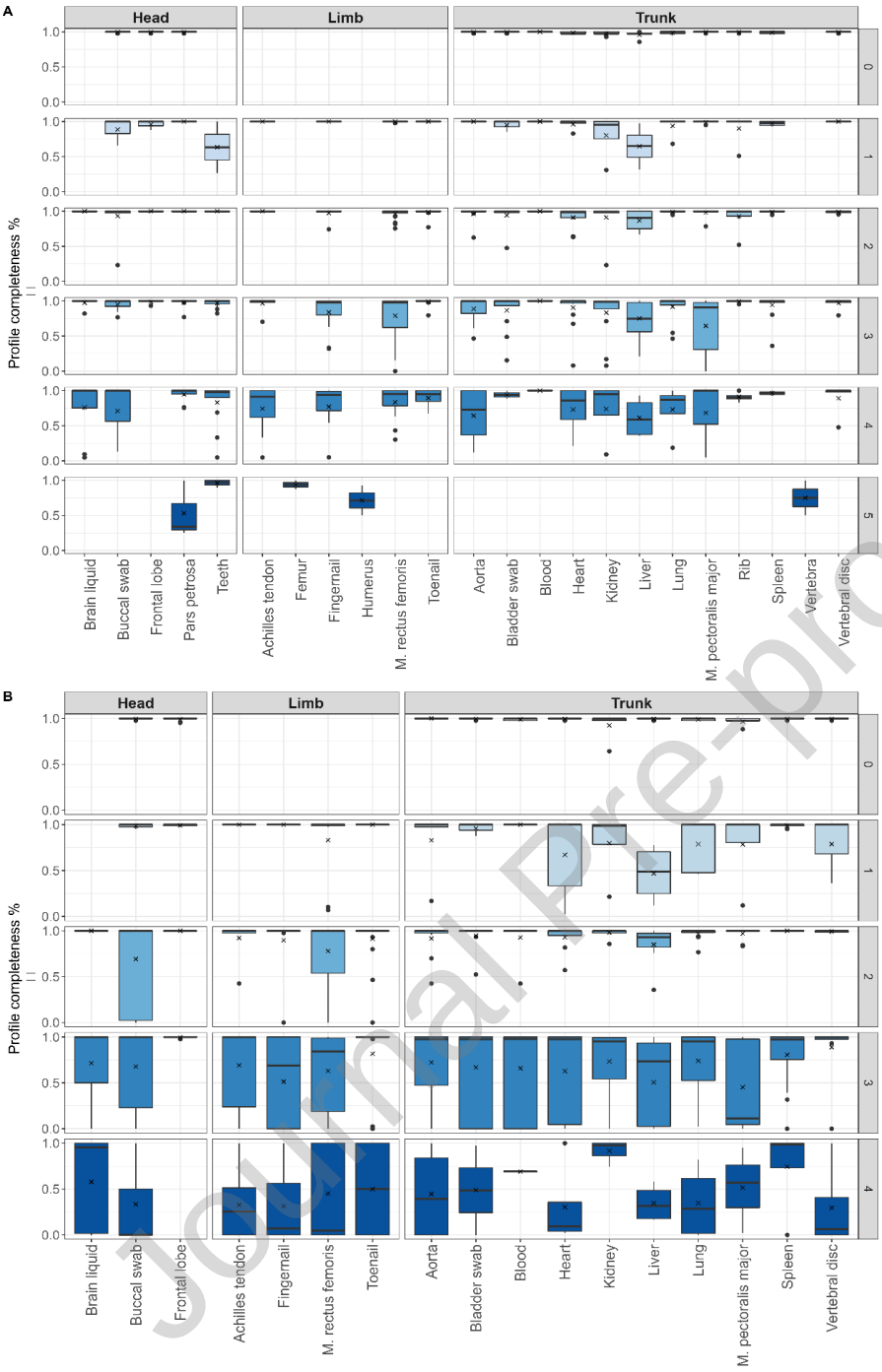

Figure 3: Profile completeness (%) of 22 STRs separated according to DNA extracted using the Maxwell® FSC DNA IQ™ Casework Kit (A) and SwabSolution™ Kit (B) from tissue samples of decomposed corpses. Samples are separated according to the corpse's degrees of decomposition from 0 (unaltered) to 5 (skeletonized) for the Maxwell® FSC DNA IQ™ Casework Kit and from 0 (unaltered) to 4 (profoundly) for the SwabSolution™ Kit. Missing data represent unavailable sample material.

For predicting the genotyping success with respect to all 22 loci, significant differences between *degree of decomposition* ($p<0.001$), *tissue types* ($p<0.001$) and *extraction method* ($p<0.001$, all p-values logistical regression) were detected. The probability of genotyping success for each tissue type and degree of decomposition is summarized in table 3 and revealed blood samples (MWK) with the highest probability of complete CE STR profiles for each degree (Tab.3). DNA extracted with the IPK/BTA method showed genotyping success rates of 100% for samples from the brain, blood, rib,

vertebral disc and femur (D_3 and D_4). The highest number of allelic dropouts was observed in a sample from the Achilles tendon D_4.

The genotyping success of samples taken from bodies found in water was similar to that of decomposed remains with > 95% of the 24Plex loci for each MWK extracted D_1 tissue, except for kidney samples (mean = 56%). For liver, lung and spleen D_3 samples, mean completeness decreased to < 78%. As for decomposed bodies, the number of allelic dropouts increased with the SSK method and mean profile completeness of liver and fingernail samples were 85% and 75%, respectively. DNA from burnt human remains only showed a decrease for D_3 samples from *M. rectus femoris* and *M. pectoralis major* (56% and 50%, MWK) and D_3 liver samples (95%, SSK).

Table 3: Probability of obtaining complete CE STR profiles of DNA samples from decomposed corpses (D_0-D_5), extracted with the Maxwell® FSC DNA IQ™ Casework and the SwabSolution™ kits and amplified with the Investigator 24Plex QS Kit. Separated are probabilities for the 22 STR loci and the 16 eESS loci with respect to each tissue and anatomical region.

Extraction method	Anatomical region	Tissue	Probability of genotyping success for 22 loci						Probability of genotyping success for eESS 16 loci					
			D_0	D_1	D_2	D_3	D_4	D_5	D_0	D_1	D_2	D_3	D_4	D_5
MWK	Head	*Pars petrosa*	0.75	1.00	1.00	0.77	0.64	0.34	0.75	1.00	1.00	0.77	0.64	0.34
		Buccal swab	0.80	0.67	0.62	0.64	0.67	NA	0.80	0.67	0.62	0.64	0.67	NA
		Frontal lobe	0.80	0.67	0.67	0.80	NA	NA	0.80	0.67	1.00	0.80	NA	NA
		Brain liquid	NA	NA	1.00	0.86	0.62	NA	NA	NA	1.00	0.86	0.57	NA
		Teeth	NA	1.00	1.00	0.61	0.46	0.50	NA	0.50	1.00	0.61	0.46	0.50
	Trunk	Aorta	0.80	1.00	0.82	0.53	0.50	NA	0.80	1.00	0.82	0.53	0.50	NA
		Bladder swab	0.80	0.67	0.70	0.62	0.50	NA	0.80	0.67	0.80	0.62	0.50	NA
		Blood	1.00	1.00	1.00	1.00	1.00	NA	1.00	1.00	1.00	1.00	1.00	NA
		Heart	0.40	0.60	0.50	0.69	0.50	NA	0.40	0.60	0.50	0.75	0.50	NA
		Kidney	0.40	0.50	0.50	0.50	0.40	NA	0.40	0.50	0.60	0.57	0.40	NA
		Liver	0.20	0.00	0.34	0.24	0.00	NA	0.20	0.00	0.34	0.29	0.00	NA
		Lung	0.60	0.80	0.80	0.53	0.25	NA	0.60	0.80	0.90	0.60	0.25	NA
		Spleen	0.60	0.50	0.75	0.69	0.25	NA	0.60	0.50	0.78	0.69	0.25	NA
		M. pectoralis major	0.80	0.80	0.91	0.24	0.67	NA	0.80	0.80	0.91	0.24	0.67	NA
		Vertebral disc	1.00	1.00	0.67	0.50	0.60	NA	0.80	1.00	0.67	0.70	0.60	NA
		Rib	0.80	0.80	0.70	0.73	0.25	NA	0.80	0.80	0.70	0.80	0.75	NA
	Limb	Achilles tendon	NA	1.00	1.00	0.70	0.43	NA	NA	1.00	1.00	0.80	0.43	NA
		Fingernail	NA	1.00	0.90	0.46	0.34	NA	NA	1.00	0.90	0.54	0.29	NA
		M. rectus femoris	NA	0.91	0.65	0.39	0.46	NA	NA	0.91	0.65	0.39	0.46	NA
		Toenail	NA	1.00	0.90	0.90	0.50	NA	NA	1.00	0.90	0.90	0.50	NA

				0	0	1	0			0	0	1	0	
SSK	Head	Buccal swab	0.80	0.67	0.62	0.64	0.34	NA	0.80	0.67	0.62	0.64	0.33	NA
		Frontal lobe	0.80	0.67	1.00	0.80	NA	NA	0.80	1.00	1.00	0.80	NA	NA
		Brain liquid	NA	NA	1.00	0.71	0.43	NA	NA	1.00	0.70	0.71	0.50	NA
	Trunk	Aorta	1.00	0.60	0.73	0.40	0.25	NA	1.00	0.80	0.73	0.40	0.25	NA
		Bladder swab	0.80	0.67	0.80	0.31	0.00	NA	0.80	0.67	0.80	0.38	0.00	NA
		Blood	0.60	1.00	0.88	0.44	0.50	NA	0.60	1.00	0.88	0.44	0.50	NA
		Heart	0.80	0.60	0.60	0.40	0.25	NA	0.80	0.60	0.70	0.38	0.25	NA
		Kidney	0.60	0.50	0.60	0.29	0.40	NA	0.60	0.50	0.60	0.29	0.40	NA
		Liver	0.80	0.00	0.11	0.06	0.00	NA	0.80	0.00	0.11	0.06	0.00	NA
		Lung	0.60	0.60	0.70	0.34	0.00	NA	0.60	0.60	0.80	0.33	0.00	NA
		Spleen	0.20	0.75	1.00	0.38	0.50	NA	0.80	0.75	1.00	0.38	0.50	NA
		M. pectoralis major	0.40	0.60	0.82	0.18	0.00	NA	0.60	0.60	0.82	0.17	0.00	NA
		Vertebral disc	0.80	0.67	0.67	0.60	0.20	NA	0.80	0.67	0.67	0.70	0.80	NA
	Limb	Achilles tendon	NA	1.00	0.62	0.60	0.14	NA	NA	1.00	0.75	0.60	0.14	NA
		Fingernail	NA	1.00	0.80	0.38	0.29	NA	NA	1.00	0.80	0.38	0.29	NA
		M. rectus femoris	NA	0.73	0.65	0.26	0.38	NA	NA	0.82	0.65	0.32	0.38	NA
		Toenail	NA	1.00	0.81	0.77	0.50	NA	NA	1.00	0.89	0.77	0.50	NA

NGS with the MiSeq FGx™ system

Read count

DNA of 155 tissue samples from decomposed corpses extracted with the MWK method was sequenced with the MiSeq FGx™ system and revealed decreasing read count for most tissue types with advanced signs of decomposition (Fig. 4). For D_1, mean numbers of reads below the recommended threshold of 85,000 [66] were only obtained from liver samples (29,207). Greater alteration of the body (D_3 and D_4) showed increased influence on soft tissues from the trunk and mean read counts below 85,000 for, among others, samples from the liver D_3 and aorta D_4 (38,086, and 2,779, respectively). Read counts were the lowest for samples D_5 from the *humerus* and *pars petrosa* (13,037, and 4,866, respectively).

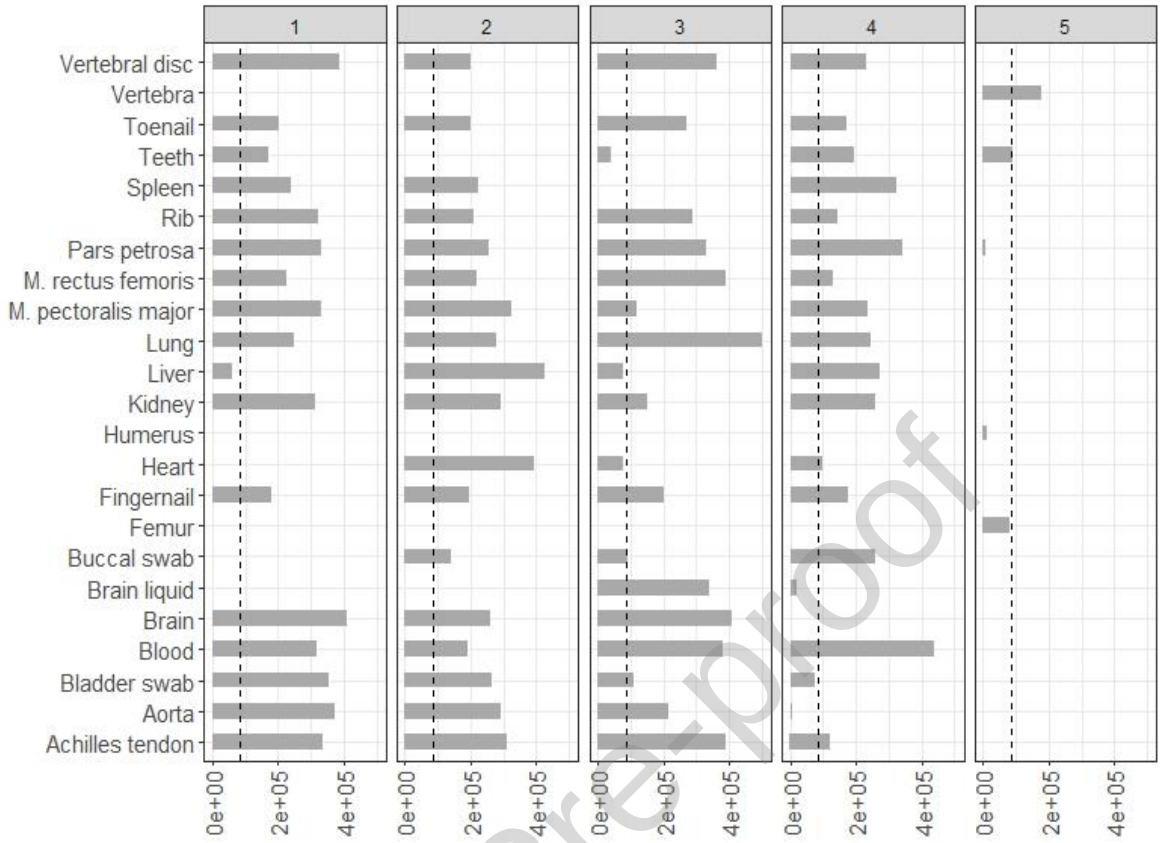

Figure 4: Read counts of samples extracted with the Maxwell® FSC DNA IQ™ Casework Kit of decomposed corpses. Samples are separated according to the corpse's degrees of decomposition and range from 0 (unaltered) to 5 (skeletonized). The dotted line represents the manufacturer's threshold of 85,000 [67]. Missing data represent unavailable sample material.

Concordance of profile completeness between CE and NGS

Statistical analyses revealed significant differences ($p<0.001$, paired Wilcoxon test) between profile completeness of overlapping loci from CE and NGS. On average, more samples (MWK) showed lower numbers of allelic dropouts with NGS (Fig. 5). However, a comparison of each degree of decomposition showed significant differences only for D_4 ($p<0.001$, paired Wilcoxon test). Samples from the heart and liquid brain samples revealed the greatest deviations with median profile completeness of 93% and 70% respectively for NGS and 73% and 48% for CE. Blood and brain samples each showed genotyping success rates of 100% for both methods.

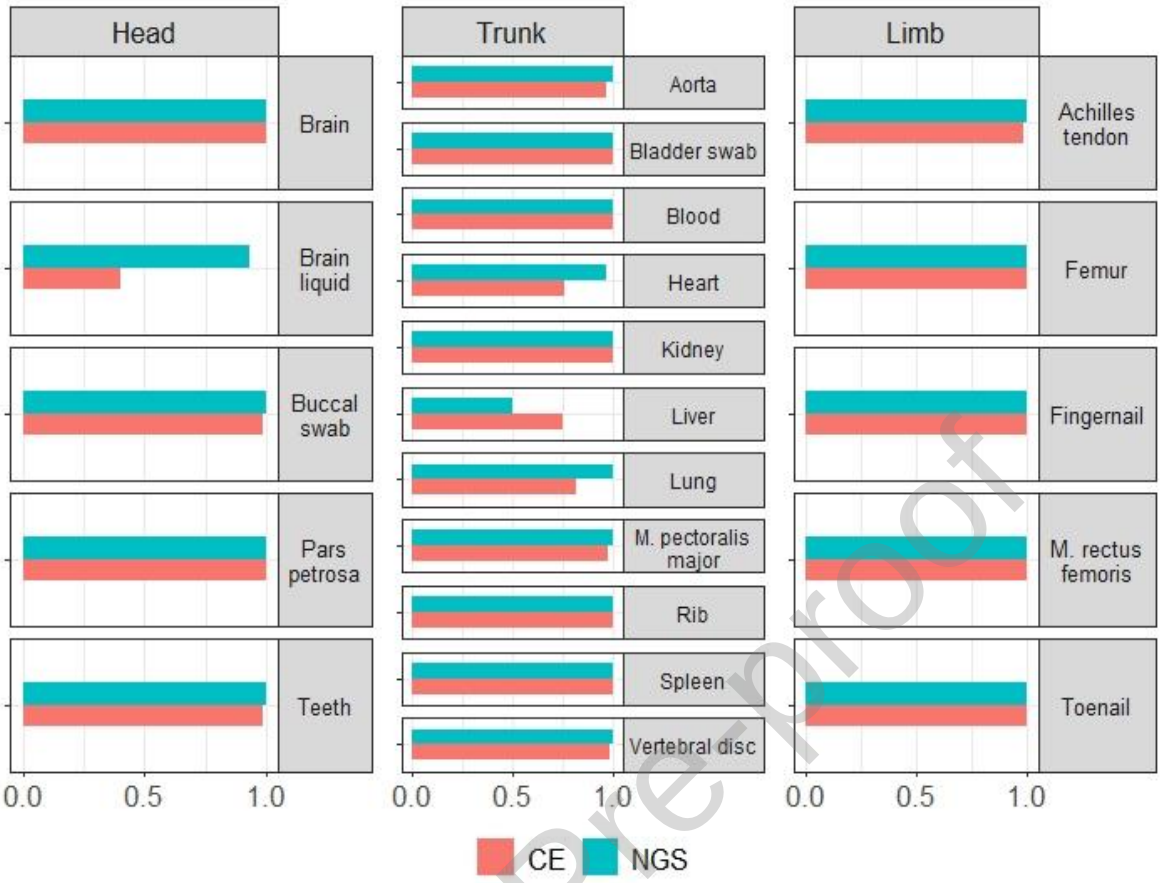

Figure 5: Profile completeness (%) of tissue samples from decomposed corpses extracted with the Maxwell® FSC DNA IQ™ Casework Kit. The results for the different degrees were summed up and the median is presented. Compared are STR profile completeness of the overlapping loci obtained with CE and NGS.

Phenotype and ancestry prediction with the MiSeq FGx™ and the Ion S5™ systems

Performance comparison of the MiSeq FGx™ and the Ion S5™ systems revealed no significant differences between the completeness of each sequenced SNP panel (piSNPs: p=0.141, aiSNPs: p=0.753, all p-values paired Wilcoxon test, Fig. 6). For piSNPs, a total of 14 samples from Achilles tendons, blood, lung, *M. rectus femoris*, ribs, vertebral disc and toenails revealed genotyping success rates of 100% for both technologies. Only one Achilles tendon sample D_4 showed distinct lower profile completeness with MiSeq FGx™ (18% with MiSeq FGx™, 43% with Ion S5™). For the tendon, similar results were observed when comparing profile completeness of aiSNP panels (30% with MiSeq FGx, 35% with Ion S5™).

Predictions of the corpses' phenotypes revealed similar tendencies for both platforms and, with the exception of one sample, concordant genotypes. For one aorta sample, genotypes differed in rs1042602, rs4959270, rs1393350, rs28777 and rs12913832. However, no deviations in hair or eye color were observed between tissue types, thus indicating the devices' reproducibility. Except for one deceased, the estimation of biogeographic ancestry was also concordant for both systems and predicted European ancestry. The exception, a highly decomposed corpse, revealed African ancestry in three out of four sequenced samples. The fourth sequence from the aorta led to a switch in ancestry estimation due to the high number of dropouts, indicating admixed American ancestry with the MiSeq FGx™ system and European ancestry with the Ion S5™ system.

Figure 6: Profile completeness (%) of tissue samples (D_2 to D_4) from decomposed corpses, extracted with the Maxwell® FSC DNA IQ™ Casework Kit. Compared are aiSNP and piSNP genotyping success rates of loci obtained with the Ion S5™ and the MiSeq FGx systems. D_1 and D_5 tissues were not available for comparison.

Discussion and Recommendations

The choice of sampling material can influence STR genotyping and is essential for a successful DNA-based identification of altered human remains. Just recently, a retrospective study has shown an uncertainty on the right sample selection, which was accompanied by parallel or sequential extra analyses, elevating time and costs [14]. Also, the large number of studies confirms the need to detect the most promising ID material for genetic profiling [3, 4, 9-12, 15, 16, 19, 20, 68]. While the prognosis for unaltered human corpses does not seem to be so demanding, material from highly degraded bodies is much more challenging or even unpredictable concerning successful STR profiling [14]. To minimize this gap, an unprecedented variety of soft and hard tissues, including the most

common ones used in ID processes, were systematically investigated within this multicenter study. Known impact factors, such as the DNA extraction methods, were covered by analyzing the influences of non-purifying and purifying systems on the ID success. As an emerging technology in forensic ID processes, NGS was included in the study, showing its valuable advantages but also limitations when compared to standard CE analysis [29]. The comparison of currently used technologies confirmed the similar power in phenotype and ancestry prognosis of human deceased as was also proven for mock samples in fairly new NGS proficiency tests [69].

The following recommendations were established for improving the first-attempt identification success of altered remains on the basis of our systematic approach and its results. Prior to sample collection, visual classification of the corpses' condition is essential. Appearances like skin discolorations, blisters or partial skeletonization can be easily identified, yet, due to highly variable environmental influences and processes of alteration within a corpse, categorizing the whole body in one score is not precise enough in most cases. Also, in our study, the TBS [32] was not suitable for categorizing the degree of decomposition of single tissue types, since the deviations observed within a score were too high. Instead, the bodies' analyzed tissues were separated into three anatomical regions and were each assigned an independent degree. This allows a faster, simpler and more precise description of possible and often diverse degradation degrees within one body.

According to the DNA quantification results, no significant differences were observed between the MWK and SSK extraction methods for decomposed remains. There was however, a tendency for higher DNA yields with SSK extractions, probably due to the capacity restriction of the magnetic beads with the MWK method [39]. Although the SSK method was developed specifically for DNA extractions from buccal swabs, it resulted in sufficient DNA yields even with challenging samples from altered remains. Accordingly, the extraction represents a cost-efficient and fast alternative in case of time shortage or financial constraints [70]. While liver and spleen samples displayed high DNA yields for both extraction methods, samples from the vertebral disc, aorta and blood revealed similar confidence intervals and higher consistency between degrees of decomposition, indicating little influence of deviations from the subjective classification of the corpse's alteration.

Besides DNA quantity, the decision of the best-suited tissue type for STR genotyping depends mainly on the quality of the extracted DNA. Allelic dropouts, imbalanced alleles and a "ski slope effect" [71] caused by DNA degradation complicate the differentiation between homo- and heterozygotes and impede the interpretation of genotyping results. Calculation of DI and the evaluation of quality sensor ratios were used to evaluate DNA integrity and revealed, concordant to the findings of Uerlings et al. [70], the highest degradation values for soft tissues extracted with the SSK method. Compared to the MWK method, DI were significantly lower in DNA extracted with the SSK method, thus the DNA was more subject to degradation. Since the sampling material was identical, lower DI observed in SSK

extracted samples are likely the result of the non-purified extraction method with inhibitors still being present. Differences between DI of tissue types were even observed for unaltered and presumably unproblematic remains (D_0), highlighting the importance of choosing the best-suited tissue for ID purposes. As expected, the ratio of the artificial sensors QS1 and QS2 showed a higher tendency of inhibitors in DNA extracted with the SSK method, which can be explained by the absence of extract purification. However, the wide ranges of quality sensor ratios and imbalances between peak heights impede the interpretation of whether inhibitors are present or not. Since no significant differences were observed between tissue types, no proposition could be made as to which tissue is more susceptible to PCR inhibition.

Due to the importance of typing as many loci as possible for identification purposes of unknown human remains, profile completeness and prognoses on STR genotyping success were presented for all 24Plex kit loci. The detected differences between tissue types and degree of decomposition emphasize the collection of sampling material according to the corpses' condition with respect to the three anatomical regions. For MWK extracted samples, D_1 samples of the trunk revealed lower profile completeness compared to D_2, which could be explained by differing sample sizes or the subjective categorization of the corpses. Interestingly, although DNA extracts were not purified with the SSK method, sufficient profiles were obtained from tissue types up to D_2 for identification purposes, indicating the robustness of the method.

The 16 *extended loci* of the *European Standard Set* are important to report profiles for an effective search in nations' databases. Since no distinct difference in probabilities of genotyping success was observed when compared to 22 loci, the following recommendations focus on the completeness of the 16 *eESS* loci, extracted with MWK method, since the majority of forensic laboratories purify expected challenging samples. Due to the smaller number of bodies found in water and burnt corpses our recommendations address only decomposed corpses. Since the sample size of bone samples D_5 was not sufficient, no guidance can be given for dry bones. However, for these materials, DVI recommendations [15] should be consulted.

Recommendation #1: *Collect a buccal swab (D_0, D_4), a sample from the frontal lobe (D_0, D_2, D_3), a sample from the pars petrosa (D_1, D_2) or teeth (D_2) from the head of decomposed corpses.*

Comparison of genotyping success of tissues collected from decomposed heads revealed brain samples with the highest probabilities of complete STR profiles for various degrees of decomposition. Those findings were also observed in the study of Uerlings et al., in which 16 out of 20 DNA samples from brain tissue extracted with the DNeasy Kit revealed complete STR profiles [70]. This could be explained by the location in the skull and the enclosure of the *dura mater*, which provides longer

protection against bacterial and insect infestation compared to soft tissues of the trunk [72]. Furthermore, according to Huang et al., the chromatin structure is properly preserved for at least 30 hours after death [73]. The results of this study also revealed bone powder from the *pars petrosa* as reliable sample material from decomposed corpses. Since the petrous bone is one of the most compact and dense bone in the human body, DNA preservation is greater compared to cancellous bones, and high DNA yields as well as low degradation can be expected [13]. According to Kulstein et. al., DNA extracted from the petrous bone led to reportable profiles in all analyzed samples [13]. Although teeth samples yielded low DNA quantities, the probability of complete STR profiles was the highest in teeth for D_2 samples, which could also be explained by a tooth's density and the enamel providing environmental protection and reduced microbial activity [26]. Unexpectedly, despite high degradation in DNA extracted from buccal swabs D_4, STR genotyping revealed the highest probability (67%) for generating complete profiles. Since the buccal collection is fast and easy and there is no damage to the body, the sampling method represents a potential source for further molecular analyses.

Recommendation #2: *Collect a blood sample (D_0-D_4), a sample from the aorta or vertebral disc (D_1) from the trunk of decomposed corpses.*

For sampling material from the trunk, blood samples are the optimal sources for STR genotyping and are recommended for unaltered and decomposed corpses ranging from D_1 to D_4. Despite advanced decomposition, small amounts of blood were still available in the heart. Even though DNA yields were comparably low, high DNA degradation indices and genotyping success rates were observed, indicating high DNA preservation and stability. Those findings are also supported by the study of Bär et al. [74]. Also, according to Shintani-Ishida et. al. [75] and Watherston et. al. [76], the DNA stability in blood is still sufficient for DNA profiling after progressive decomposition. Additionally, the best-suited tissue samples from decomposed corpses D_1 are samples from the aorta and vertebral disc. Concordant with the results of Sato et al. [77], aorta tissue represents a promising source for STR genotyping. This could be explained by the resilient and elastic structure of the aortic wall, making it more resistant to decomposition processes [72]. Furthermore, as also shown in the study of Becker et al. [4], samples from the vertebral disc revealed high quality DNA profiles, which could be explained by the cells being embedded in an extracellular matrix, making them less susceptible to decomposition processes.

Compared to other soft tissues from the trunk, liver samples severely underperformed for each degree of decomposition and are therefore not recommended for DNA-based identification. In line with the findings of Uerlings et al. [70], Schwark et al. [17] and Helm et al. [72], liver samples revealed high DNA degradation and low profile completeness, which could be explained by the large number of lysosomes facilitating post mortem destruction of the cell membrane [70].

Recommendation #3: *Collect a sample from the Achilles tendon (D_1, D_2), fingernail (D_1), or toenail (D_1, D_3, D_4) for limbs of decomposed corpses.*

A comparison of tissue types taken from limbs revealed the Achilles tendon as optimal sampling material. Supported by the study results of Roeper et al. [10], high STR genotyping success is observed, indicating profound DNA stability and protection against autolysis and putrefaction. Furthermore, nails are recommended as best-suited sampling material from corpses classified D_1, D_3 and D_4. Finger- and toenails are robust, available from bodies with wide ranges of alteration, and can be easily removed when limbs are not mummified or dried [10]. The greater DNA stability might be explained by the protected location, not only because DNA adheres to the underside of the nail and the nail bed but also because it is preserved within the keratin structure [76]. Since no opening of the body is necessary, nail samples can be collected even if no autopsy is ordered or if manipulation of the body should be avoided due to religious reasons.

Recommendation #4: *Combination of recommended tissue types*

As the provided classification is divided into three anatomical regions of the corpse, up to three different degrees of decomposition or burning are possible. For example, a decomposed corpse categorized D_1 (head), D_3 (trunk) and D_4 (limbs) would lead to a sampling recommendation of *pars petrosa*, blood and toenail samples. In order to collect the best-suited material, the individual case and the availability of sampling material (in case of missing body parts) has to be considered. Also, the integrity of the body has to be considered, especially if no autopsy is ordered or not feasible due to religious reasons, the body cannot (or should not) be opened and only minimal invasive alteration of the body can be executed. Considering the probability of the genotyping success, table 3 provides guidance on the most promising material for human ID at the first attempt. For example, *pars petrosa* from the D_1 head, blood from a D_3 trunk and toenails from D_4 limbs regions, will lead to 100% (head, blood) and 50% (toenail) profiling success, respectively. In short, the most promising region and tissue are trunk and blood, regardless of the degree of decomposition.

Recommendation #5: *Consider NGS for identification*

The growing demands in human identification require constant improvements in methods of analysis, as shown for the NGS application. Here, the study results displayed the sequencing technology as a reliable and promising method for improving the ID success of altered human remains, exceeding CE STR genotyping as demonstrated in a recent study [26, 27, 29]. Assessment of tissue-specific

differences revealed higher STR profile completeness with NGS for samples from the heart, vertebral disc, and Achilles tendon, while lower profile completeness could be found in liver samples. The lowest read counts for dry bone samples D_5 could be explained by lower DNA yields, individual DNA variations, the age of the bones and small sample sizes. Observed genotyping success rates of 100% in blood and brain samples for both CE- and NGS-based genotyping could also be explained by the location of the brain within the skull and increased preservation and stability. For countries who currently undergo law revisions (or might in the future) with respect to phenotype and biogeographic ancestry prognoses, the evaluation of reliable methods of analysis is increasingly important. Our research results showed a similar performance power in profile completeness and correctness of SNP panel, with both the MiSeq FGx™ and the Ion S5™ systems. However, caution is advised when interpreting sequencing data of degraded DNA samples. As observed for one sample of the aorta, high numbers of allelic dropouts can lead to a switch in the estimation of biogeographic ancestry for both sequencing technologies.

Conclusion

The presented recommendations for improving the identification success rates of altered human remains at first attempt can be implemented directly at the intersection of forensic medicine and forensic genetics. Since the assessment of the corpse´s condition is crucial for STR genotyping, the presented categorization system should be applied prior to the sample collection in order to accurately score the progress of alteration. Here, the classification is based on visual post-mortem characteristics, including skin discoloration or blisters that can be reliably identified and described by the examiner. Depending on the determined degree of decomposition, the probability of STR profiling success in table 3 can be used as direct guidance to select the most promising tissue types for successful genetic downstream analysis, separated for the necessary STR loci and DNA extraction method.

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Highlights

- Systematic approach of a broad variety of soft and hard tissues for human identification
- Novel categorisation in three anatomical regions of corpse prior to sampling
- Blood samples outperformed all other tissues, regardless of decomposition degree
- Higher STR profile completeness in NGS than in CE genotyping
- Novel recommendations for alteration-specific optimal tissues for first-attempt identification