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Extraction of DNA from decomposed human tissue An evaluation of five extraction methods for short tandem repeat typing

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Abstract

Hyperpolymorphic short tandem repetitive DNA sequences, STRs or microsatellites, have become widely used in human identification, particularly in criminal cases and in mass disasters. In such cases the substrates for the analyses may be decomposed biological material, a fact that has to be taken into account when choosing the appropriate casework methods. In this paper we report the evaluation of five different DNA extraction methods, namely the phenol–chloroform, the silica based, the InstaGene Matrix™ (BioTest), the glass fiber filter, and the Chelex based methods. The substrates for the analyses are decomposed human liver tissue specimens from forensic autopsy cases. Extracted DNA was quantified and DNA profiled by a set of seven STRs. We have compared laboratory time consumption and costs of the five methods, showing that the Chelex method is the more rapid and less expensive of the methods, the phenol–chlorophorm and silica extractions being the most time consuming and resource demanding ones. A full profile was obtained by the silica method in nine out of ten cases and this method failed to give a reliable type in four out of 70 STR analyses. The phenol–chlorophorm and the glass fiber filter methods failed in 16 analyses, the InstaGene Matrix™ (BioTest) in 25 and the Chelex extracts in 56 of the 70 STR analyses. By multiple logistic regression we show that the difference between the silica procedure and the other methods are statistically significant. In our hands, the silica gel extraction procedure is an obvious choice when the biological material available is decomposed human tissue

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— even if this procedure is one of the more laborious ones.

Keywords: Decomposed tissue; DNA extraction methods; STR analyses

1. Introduction

Hyperpolymorphic short tandem repetitive DNA sequences, STRs or microsatellites, have become widely used in human identification, particularly in criminal cases [1–3] and in mass disasters [4,5]. In such cases the substrates for the analyses may be decomposed bodies [6], a fact that has to be taken into account when choosing the appropriate casework methods, such as extraction procedures.

STR typing for forensic purposes have proven to be successful even under extreme sample conditions [4,6,7]. After the 1996 Spitsbergen aircraft disaster, victim bodies and body parts were removed from the scene up to 9 days after the disaster. This fact did not hamper the DNA based sorting of body parts and identification of all victims for whom reference samples were available [5]. These studies all utilized an organic DNA extraction procedure only.

The laboratory performing DNA analyses for identification purposes may for obvious reasons be put under time pressure from the relatives of missing persons or the law enforcement authorities requesting the analyses. This calls for the evaluation of the time consuming aspects of the different methodological steps in the laboratory.

Due to the implementation of polymerase chain reaction (PCR) based methods, the successful extraction of high molecular weight DNA molecules is not longer a prerequisite for satisfactory electrophoretic results. Alternatives should therefore be sought for to traditional organic methods like the relatively time consuming phenol–chloroform extraction [8]. Potentially health hazardous chemical reagents should be avoided.

In this report we present a comparison of five different methods of extracting DNA from decomposed human soft tissue. We have compared the time required to perform the extraction, the cost of the method and the quality of the electrophoretic products. For all samples seven STRs were amplified, several of which are in widespread forensic use.

In addition to phenol–chloroform extraction we have evaluated the organic silica method [9,10]. We have also chosen the inorganic methods InstaGene Matrix™ from BioTest, the glass fiber filter paper procedure [11] and the chelating resin Chelex 100 [12,13].

2. Materials and methods

2.1. Materials

Ten consecutive legal autopsy cases with some degree of tissue decomposition were chosen for this study. The ascertainment of the degree of decomposition was based on

the pathologists description of the actual signs of decomposition, i.e., discoloration, ''marbling'' or blistering of the skin, swellings, maggot infestation or putrefaction of internal organs. A scale for the degree of decomposition ranging from 1+ to 4+ was constructed. The material is presented in Table 1.

In each case an approximately 2.0×2.0×3.0 cm piece was cut from the liver at autopsy. Each specimen was immediately frozen at −20°C until extraction and further analyses. A compact bone sample (femur shaft) was included for DNA type verification purposes.

The amount of tissue substrate for each extraction procedure was determined according to the guidelines for each procedure. For the glass fiber filter method and the InstaGene Matrix™ (BioTest) method specific guidelines in the literature [11] and in the information from the manufacturer were met, respectively. For the three other methods, the current guidelines of our routine laboratory were met. According to this, no attempts were made to equalize the amount of tissue substrate for the five extraction procedures.

2.2. Methods

2.2.1. DNA extraction

2.2.1.1. Phenol–chloroform extraction (modified after Sambrook et al. [8]) Five ml of an extraction buffer of pH 8.0 (10 mM Tris–HCl, 10 mM EDTA, 100 mM NaCl, 2% sodium dodecyl sulfate) was added to the approximately 1.0 cm^3 tissue sample before gentle mixing for 30 min at 37°C. Fifty μl proteinase K (20 μg/ml) was added followed by gentle mixing at 37°C overnight. Ten ml chloroform–phenol–isoamylalcohol (49.5:49.5:1.0) was added and the solution was centrifuged at 3500 rpm for 10 min. The upper layer was transferred to another tube and mixed with 5 ml chloroform–phenol–isoamylalcohol followed by a new centrifugation. The upper layer was again transferred and mixed with 5 ml chloroform isoamylalcohol (24:1) before the third centrifugation at 3500 rpm for 10 min. The supernatant was again transferred to another tube and DNA made insoluble by the addition of two to three times the solution's volume of cold absolute ethanol to the supernatant. This solution was then centrifuged in a cold environment at maximum speed for 30 min. DNA was rinsed by centrifugation in 70% alcohol at maximum speed in a cold environment and subsequently dried up before it was made soluble in 50 μl of a TE buffer of pH 7.6 (10 mM Tris, 1 mM EDTA). The solution was then shaken overnight.

2.2.1.2. Silica extraction (modified after Höss and Pääbo [10]) In preparation of a silica suspension ([9]), 60 g of silica and water were added up to 500 ml. The solution was then left for 24 h at room temperature, 430 ml of the supernatant removed and water added again up to 500 ml. The solution was well shaken to distribute the silica particles. The solution was left for 5 h at room temperature, 440 ml of the supernatant was removed and finally 600 μl of concentrated HCl was added to the solution.

Two ml of an extraction buffer (10 M guanidine isothiocyanat [GuSCN], 0.1 M Tris–HCL of pH 6.4, 0.02 M EDTA of pH 8.0 and 1.3% Triton-X) was added to the approximately 1.0 cm^3 liver sample before incubation and gentle agitation mixing at

Table 1
Description of the material and electrophoretic results (see text for details)

Case#	Scene	PM time (days)(a)	Decomp	STR	APO	D11	SE33	THO	FES	VWA	F13	Total	Total
				METHOD								(7 STRs)	(5 methods)
1	Lake	90	Yes(b)	Phenol(c)	+	+	+	+	-	+	-	5	
				Silica	+	+	+	+	+	+	+	7	
				Glass fiber(d)	+	+	+	+	-	+	-	5	
				InstaGene(e)	+	-	-	+	-	-	-	2	
				Chelex	-	-	-	-	-	-	-	0	19
2	Home	2	2+	Phenol	+	+	+	+	+	+	+	7	
				Silica	+	+	+	+	+	+	+	7	
				Glass fiber	+	+	+	+	+	+	+	7	
				InstaGene	+	+	+	+	+	+	+	7	
				Chelex	-	-	-	-	-	-	-	0	28
3	Home	25	2+	Phenol	+	+	+	+	+	+	+	7	
				Silica	+	+	+	+	+	+	+	7	
				Glass fiber	+	+	+	+	+	+	+	7	
				InstaGene	+	+	+	+	+	+	+	7	
				Chelex	-	-	-	+	-	+	-	2	30
4	Home	27	3+	Phenol	-	-	-	-	-	-	-	0	
				Silica	-	-	-	+	+	+	-	3	
				Glass fiber	+	-	-	-	-	-	-	1	
				InstaGene	-	-	-	+	-	+	-	2	
				Chelex	-	-	-	-	-	-	-	0	6
5	Home	5	2+	Phenol	+	+	+	+	+	+	+	7	
				Silica	+	+	+	+	+	+	+	7	
				Glass fiber	+	+	+	+	+	+	+	7	
				InstaGene	+	+	+	+	+	+	+	7	
				Chelex	-	-	-	-	-	-	-	0	28
6	Home	15	4+	Phenol	+	+	+	+	+	+	+	7	
				Silica	+	+	+	+	+	+	+	7	
				Glass fiber	+	+	+	+	+	+	+	7	
				InstaGene	+	+	+	+	-	+	+	6	
				Chelex	+	+	+	+	+	+	+	7	34
7	Home	16	4+	Phenol	+	+	+	-	-	-	-	3	
				Silica	+	+	+	+	+	+	+	7	
				Glass fiber	+	+	+	+	-	+	-	5	
				InstaGene	-	-	-	-	-	-	-	0	
				Chelex	-	-	-	-	-	-	-	0	15
8	River	17	4+	Phenol	+	+	+	+	+	+	+	7	
				Silica	+	+	+	+	+	+	+	7	
				Glass fiber	+	+	+	+	+	+	+	7	
				InstaGene	+	+	+	+	+	+	+	7	
				Chelex	-	-	-	-	-	-	-	0	28
9	Home	11	4+	Phenol	+	+	+	+	+	+	+	7	
				Silica	+	+	+	+	+	+	+	7	
				Glass fiber	+	+	+	+	+	+	+	7	
				InstaGene	+	+	+	+	+	+	+	7	
				Chelex	+	+	+	+	-	+	-	5	33
10	Home	7	3+	Phenol	-	-	-	+	+	+	+	4	
				Silica	+	+	+	+	+	+	+	7	
				Glass fiber	+	-	-	-	-	-	-	1	
				InstaGene	-	-	-	-	-	-	-	0	
				Chelex	-	-	-	-	-	-	-	0	12
Total number of successive profiles per STR					36	33	33	37	29	36	29		

(a) Number of days from the assumed time of death to the autopsy
(b) No description of the degree of decomposition in the autopsy report
(c) Short for phenol-chlorophorm extraction method in all tables herein
(d) Short for glass fiber filter extraction in all tables herein
(e) Short for the InstaGene Matrix™ (BioTest) in all tables herein

60°C for at least 3 to 4 h, alternatively overnight. The solution was then centrifuged at 6000 rpm for 5 min. Six hundred μl were removed from the supernatant and added to 400 μl of the extraction buffer and 40 μl of the silica suspension.

For binding of the DNA to the silica particles, the solution was left for 10 to 30 min at room temperature and then centrifuged for 3 min at maximum speed before removing the supernatant. To avoid the formation of potentially health hazardous by-products, the supernatant was stored in 10 M NaOH until safe destruction. The silica pellet was then washed: two times in 750 μl of a washing buffer at pH 6.4 (10 M GuSCN, 0.1 M Tris–HCl), two times in 750 μl of 70% ethanol and once in 750 μl of acetone. The pellet was dried at 56°C. The DNA was eluted two times with 65 μl TE, each time in 10 min at 56°C.

2.2.1.3. Glass fiber filter extraction. This method was applied as described by Jiang and Lee [11]. The liver tissue specimen was rinsed with 1 ml of proteinase K buffer (2.5 ml 1 M KCl, 750 μl 1 M Tris–HCl at pH 8.3, 2.5 ml 10% Igepal CA-630 detergent (ICN Biomedical Inc.), dH_2O added up to 50 ml). The specimen, of maximum 0.3 cm^3, was then placed on a circular glass fiber filter of diameter 0.7 cm (Schleicher and Schuell Inc., filter grade 50) and the filter containing the tissue was soaked in 100 μl of methanol for 10 min in a microcentrifuge tube. The methanol was removed by suction and the microcentrifuge tube with the filter were dried in a heat container at 80°C for 15 min. If necessary, the inner wall of the microcentrifuge tube was dried with a cotton swab. A 120 μl volume of the proteinase K buffer with 500 μg/ml proteinase K was added to the tube and this was incubated at 55°C for 45 min. The proteinase K was inactivated by boiling the tube in water for 10 min. The tube was then centrifuged for 10 s and 10 μl of a total of 100 μl clarified supernatant was used for PCR amplification.

2.2.1.4. InstaGene Matrix™ (BioTest) extraction. This extraction procedure was performed as communicated by the manufacturer: A 0.3 cm^3 piece of liver tissue was macerated slightly with a clean scalpel and then placed in a 1.5 ml sterile screw cap Eppendorf tube. One ml 1×Trypsin–EDTA (Gibco BRL) was added and the solution was incubated at room temperature for 15–30 min with occasional shaking. After spinning down (13,000 rpm, 2–3 min), all supernatant was carefully removed. A 250 μl volume of InstaGene matrix (BioTest) was added and the tube was well mixed before incubation with occasional shaking at 56°C for 30 min. The tube was then vortexed for 10 s and placed in a boiling water bath, alternatively in a 100°C heat block for 8 min. After another vortex for 10 s, the tube was centrifuged at 13,000 rpm for 2–3 min and the supernatant removed to another Eppendorf tube. Twenty μl was used for each PCR reaction.

2.2.1.5. Chelex extraction (modified after Walsh et al. [13]). A 0.3–0.5 cm^3 piece of liver tissue was chopped finely with a clean scalpel and then placed in a 1.5 ml sterile Eppendorf tube. The specimen was then macerated, either by use of a disposal pestle or pipette with the end of the pipette removed, in 50 μl of sterile water. Thereafter, 150 μl 20% Chelex, 2 μl of 10 mg/ml protein kinase and 7 μl 1 M dithiothreitol (DTT) was

added and well mixed. The solution was incubated at 56°C for at least 30 min, alternatively at 37°C overnight.

After piercing the cap of the tube with a heated disposable needle the tube was boiled for 8 min. A sticky label was then used to seal the puncture hole before microcentrifugation at 13 K for 3 min. The supernatant was then removed to another 1.5 ml Eppendorf tube and rinsed in 20% Chelex.

2.2.2. Preparation of bone control samples

In each case an approximately 5 cm^3 rectangular piece of the femur shaft was cut with a saw. Soft tissue was removed with a scalpel before freezing the sample at −20°C. Clean bone was exposed by use of a coarse sandpaper under running water while the sample was thawing. The bone was dried with a paper towel and subsequently clamped in a small vice. A 0.3–0.4 cm thick section (weight approximately 1 g) was cut off using a clean hacksaw blade. The bone fragment was powdered in a Spex 6700 Freezer Mill (Glen Creston, UK) and then stored at −20°C.

All bone samples were subjected to DNA extraction by the silica method (Section 2.2.1.2). About 0.5–1.0 g of bone powder was added to 0.2 ml of the extraction buffer.

2.2.3. DNA quantitation

The samples were quantified by a semiquantitative dot-blot system, QuantiBlot™ (Perkin Elmer), using chemiluminescence detection as detailed by Walsh et al. [14].

Samples that gave ≥5.0 ng/μl of DNA as measured with this quantitation system are defined as abundant (A). The samples that gave between 0.1 and 5.0 ng/μl are defined as detectable (D), and samples that gave ≤0.1 ng/μl are defined as not detectable (−).

2.2.4. PCR conditions and electrophoresis

Seven STRs used in our routine forensic casework were chosen for this study. Four of the markers are included in the widely used Quadplex, namely HUMF13A1, HUMTH01, HUMVWA31/A and HUMFES/FPS [15]. The three others are the hyperpolymorphic loci HUMAPOAI1 [16,17], D11S554 [18] and HUMACTBP2 (SE 33) [19].

The markers HUMTH01 and HUMVWA31/A display alleles in the size range from 138 to 174 base pairs, the five other markers as a group have alleles in the range from 176 to 333 base pairs. The PCR conditions were as earlier communicated [20,21].

The PCR products were subjected to denaturing gel electrophoresis on an ABD Prism™ 377 gene sequencer using recommended conditions as described [21]. Genescan 672 software (Applied Biosystems Inc.) was applied for fragment analyses.

A PCR product was scored as an allele if the electrophoretic peak height was greater than 50 units relative fluorescence measured on an arbitrary scale on the *x*-axis. An exception from this principle is a ''stutter band'', i.e., a PCR product less than 11% of a four base pair larger allele [22], which was not scored as an allele even if its peak height exceeded this level.

2.2.5. Statistical methods

The statistical analysis was based on standard odds-ratio (OR) calculations. Multiple logistic regression was performed to check the validity of the OR calculations controlling for possible confounders. The chi square test was also included in the analyses. The methods and their implementation in the statistical programme SPSS is explained in Ref. [23].

3. Results

3.1. Extraction yields

The DNA yield for all five extraction methods are summarized in Table 2.

In all cases except case 4 abundant DNA was achieved by the phenol–chloroform and the silica extraction methods. In this case the phenol–chloroform and silica extraction yields were lower but detectable. The degree of decomposition was extensive (Table 1).

Given the fact that the amount of tissue available for extraction was in accordance with the protocol for each extraction method, the data given in Table 2 indicate that the InstaGene Matrix™ (BioTest) method gives better results than the glass fiber filter method. The glass fiber filter method seems to give better results than the Chelex method.

3.2. Time consumption

The time needed to extract DNA by each method was measured by summation of the time consumed by all steps in the laboratory, including incubation periods. The results of these measurements are depicted in Table 3.

The extraction methods may thus be divided into two groups in terms of the time consuming aspect: The phenol–chloroform and the silica methods are far more time consuming than the three other methods. The phenol–chloroform as well as the silica method take from 33.5 to 35.0 h more per sample than the other methods. The glass fiber filter, InstaGene Matrix™ and Chelex method display practically identical and by far shorter laboratory preparation times.

Table 2
Semiquantitative DNA yield in five different extraction methods[a]

Extraction method	Case									
	1	2	3	4	5	6	7	8	9	10
Phenol	A	A	A	D	A	A	A	A	A	A
Silica	A	A	A	D	A	A	A	A	A	A
Glass fiber	D	D	A	−	D	D	D	D	D	−
InstaGene	D	A	A	−	D	D	D	A	A	D
Chelex	−	−	D	−	−	D	−	−	D	D

[a] A: Abundant. D: Detectable. −: Not detectable.

Table 3
Laboratory time (h) for five different extraction methods

Extraction method	Total preparation time (h)
Phenol	36.0
Silica	36.0
Glass fiber	2.5
InstaGene	1.5
Chelex	1.0

3.3. Cost of the methods

The financial cost of each method was roughly calculated by the summation of the current local prices of both the different chemical solutions specific to each method and of the commercial kits utilized. Stock chemical solutions, disposable parts or the electricity consumption were not included in the calculations. The cost of the laboratory labor for each method was measured by the time spent for one skilled laboratory assistant to perform each extraction procedure. These two variables are depicted in Table 4.

As shown in Table 4, pairwise comparisons between the total cost of equipments and the expense of labor for each method are fairly consistent. The methods that show the highest costs for equipments also have the highest wage costs. Thus, the methods may be divided into two relatively ''high cost'' methods, namely the phenol–chloroform and the silica method, two ''medium cost'' methods, that is the glass fiber filter and the InstaGene Matrix™ method and finally, the relatively ''low cost'' Chelex method.

3.4. Electrophoretic results

To resemble routine forensic casework, the electrophoretic results after the different extraction procedures were evaluated on the basis of one electrophoretic run only, that is each primary standard electrophoresis performed. The term ''typeable electrophoretic result'' in this context means electrophoretograms with alleles (as defined in Section

Table 4
The laboratory preparation cost (USD) per sample and working time (min) for five different extraction methods[a]

Extraction method	Total cost (USD)	Working time per extraction (min)
Phenol	5.30	30.0
Silica	2.00	30.0
Glass fiber	0.50	8.3
InstaGene	1.30	8.3
Chelex	<0.10	5.5

[a] USD=US dollars.

Table 5
Typeable (+) vs. nontypeable results (−) with five different extraction methods

Extraction method	+	−
Phenol	54	16
Silica	66	4
Glass fiber	54	16
InstaGene	45	25
Chelex	14	56

2.2.4) known from our population database. Furthermore, the actual allotype has to match the control specimen, in this case silica extracted bone tissue. Bone tissue gave STR profiles for all cases. All liver tissue profiles matched the corresponding bone profile.

Table 1 displays the electrophoretic results after the five different extraction procedures. ''+'' And ''−'' denote a typeable vs. a nontypeable result for the STR in the corresponding column. The gray background color indicates a full profile, that is a typeable result in each of the seven STRs. As depicted in Table 1, the silica method gives nine out of ten cases with a full profile. Both the phenol–chlorophorm and the glass fiber filter methods give six out of ten, the InstaGene matrix gives five out of ten and the Chelex method one out of ten cases with a full profile.

The silica method failed to give a reliable type in four out of 70 STR analyses. These four analyses all were in case 4. The phenol–chlorophorm and the glass fiber filter methods failed in 16 analyses, the InstaGene Matrix™ (BioTest) in 25 and the Chelex extraction in 56 of the 70 STR analyses (Table 1).

The data used for OR calculations are derived from Table 5.

Table 6 summarizes the statistical results. The silica method is the reference category. From the rightmost column, all four methods are seen to differ significantly from the silica procedure. The *P*-values remain significant at the 5% significance level if Bonferroni corrections are applied. Exactly the same *P*-values are obtained by using the chi square test.

The total number of successive profiles per STR is also displayed in Table 1. The two STRs with the shortest alleles, that is HUMTH01 and HUMVWA31/A, produce 37 and 36 successive profiles out of 50 possible, respectively. The numbers for the two STRs in the Quadplex with longer alleles, that is HUMF13A1 and HUMFES/FPS, are 29 for both. The three hyperpolymorphic markers HUMAPOAI1, D11S554 and

Table 6
Results of the odds-ratio (OR) calculations

Variable	OR	95% Confidence interval	*P*-Value
Phenol	0.21	0.07–0.65	0.004
Glass fiber	0.21	0.07–0.65	0.004
InstaGene	0.11	0.04–0.34	<0.001
Chelex	0.02	0.01–0.05	<0.001

HUMACTBP2, which all belong to the group with longer allele lengths, display 36, 33 and 33 successive profiles, respectively.

3.5. Overall successfulness of DNA profiling

The successfulness of the DNA profiling of the specimens, independent of the extraction method used, was measured by counting all typeable electrophoretic results in each case. Maximum successfulness is achieved by typeable electrophoretic results after five different extraction procedures in seven STR systems. Thus, maximum successfulness is quantified to the number 35.

As shown in Table 1, cases 6 and 9 both have high decomposition scores (both 4+) and both give high scores of successfulness, i.e., 34 and 33. Cases 4 and 10 both have lower decomposition scores (i.e. +++), but give the lowest scores (i.e. 6 and 12, respectively) for DNA profiling successfulness. On the other hand, cases 2, 3 and 5 all have relatively low decomposition scores (i.e. ++) and relatively high scores of successfulness (i.e. 28, 30 and 28, respectively).

4. Discussion

The present work on different DNA extraction procedures was conducted to improve the routine forensic laboratory service in cases involving the analyses of degraded human tissue. This topic has in particular reference to human identification, either of single deceased individuals or of mass disaster victims.

In our experience, the liver is generally an organ displaying signs of putrefaction and hence tissue decomposition relatively early in the postmortem period. Liver tissue is considered a difficult substrate in terms of DNA analyses of a decomposed corpse. We therefore chose this organ as substrate for the DNA analyses in this study. We have not attempted to give exact measures on the degree of decomposition. However, the present quantification of the signs of decomposition (Table 1) was chosen since it is easily applicable in practical casework.

Whether a full STR profile is achieved is considered a crucial parameter in forensic casework. We have therefore chosen this parameter in the evaluation of the methods.

Several studies have shown that degraded tissue may be the substrate for the DNA based identification of a dead body or its remains [4,6,7]. Our results on the overall successfulness of DNA profiling (Table 1) indicate that the visual impression of the degree of decomposition of a human corpse or tissue specimen gives poor predictions as to the successfulness of the subsequent PCR based DNA profiling, irrespective of the extraction method utilized.

The STRs utilized in this study may be divided into two groups in terms of average allele lengths; the markers HUMTH01 and HUMVWA31/A display shorter alleles than the five other markers. Albeit statistically nonsignificant results, our data may lend some support to earlier observations [6,24] that markers with shorter allele lengths seem to amplify easier when the substrate is degraded (Table 1).

The phenol–chloroform and silica extraction methods resemble each other concerning the following points:

- they are both relatively time consuming (Table 3).
- they both include the use of potentially health hazardous chemicals (i.e. phenol, chloroform and GuSCN, respectively).
- they both give abundant DNA substrate (Table 2) when using the amount of tissue according to the protocols.

There are, however, certain differences. The silica based method is less expensive than the phenol–chloroform method. Most important, the silica method gives a full STR profile in 90% of the cases, the phenol–chloroform in 60%. This fact makes the silica procedure the method of choice. Others have shown that this extraction works well on extremely old and degraded bone material, both human [25] and of another species [10].

Statistical evaluation of the data for successful typing vs. a nontypeable result (Tables 5 and 6) shows that the differences between the silica procedure and the other methods are statistically significant. Multiple logistic regression analysis controlling for different bodies (results not quoted) leads to lower *P*-values, confirming the validity of the simpler analysis reported.

The glass fiber filter method, the InstaGene Matrix™ and the Chelex method only show minor differences in terms of both the time consumed and the cost of the method (Tables 3 and 4). The glass fiber filter method gives a full STR profile in 60% and the InstaGene Matrix™ in 50% of the cases (Table 1). However, in our hands the glass fiber filter method is much more ''tricky'' and troublesome to perform and therefore seems to demand a higher degree of technical skill than the InstaGene Matrix™ method.

The Chelex method only produced one full profile and gave no results in seven out of ten cases (Table 1). Sweet et al. [26] demonstrated that the Chelex method was more effective than phenol–chloroform in extracting DNA from saliva. On the other hand, dealing with extremely old, degraded tissue, Höss and Pääbo (Ref. [10] and a reference therein) demonstrated that the Chelex method failed in extracting amplifiable DNA. This observation and our present data indicate that the Chelex method may be sensitive to PCR inhibitors in degraded tissue samples.

Several reports on PCR based DNA profiling following mass disasters of various kinds report the use of organic methods to obtain DNA [4,5,27,28]. The present data give strong indications that the silica based method should be used when body decomposition is encountered.

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