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"UNUSUAL" TISSUES AND SAMPLE COLLECTION STRATEGIES ON EXHUMED BODIES

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ABSTRACT

The choice of soft or hard tissues to be sampled in case of exhumation of corpses for identification purposes or family relationship testing is based on the degradation conditions of the corpse: the more the corpse is degraded, the less DNA is expected to be retrieved from soft tissue. Therefore, the choice of the "best" tissue samples usually falls on teeth and bones in these "difficult" cases, even though the DNA extraction procedure requires time and effort and it can often result in unexpected, negative results.

We here present the results of a daily practice survey that shows that it is possible to obtain good results even on DNA extracted from tissues that appear to be less "appealing" to the examiner by performing "simple" corneal/scleral swabs along with cartilage.

While DNA extracted from cartilage has been already described, to our knowledge there is no evidence of publications in the scientific literature dealing with cornea/sclera as a source of DNA in the forensic laboratory.

The obtained results demonstrate that it may be advisable to consider other tissues which bear the potential of returning good profile results despite not appearing particularly useful and better control of contamination.

1. Introduction

DNA-STR analysis is the method of choice in the genetic characterization of human remains, either for identification purposes or for family relationship issues [1].

DNA extracted from human remains, as of quantity and quality, is the result of the decay process which takes place on cadavers and finally leads to the dissolution of soft tissues [2].

It is common experience to observe that the higher the degradation of the soft tissue, the lower the DNA yield. This forces the analyst to shift to the collection of harder tissues which can offer more choice of preserving DNA [3] with the disadvantage of requiring tedious and time-consuming extraction procedures.

The DNA extraction procedures performed on hard tissues frequently require preliminary mechanical steps aimed at assuring an appropriate grade of tissue fragmentation (e.g. liquid nitrogen, drills, mortar/pestles, etc.) followed by a decalcification phase. The tools used and the number of steps the samples go through, bear the disadvantage of a higher risk of contamination when compared to other softer tissues [4].

Therefore, we focused our attention on biological tissues which could be able to provide similar if not better results in terms of DNA amount using fewer complex procedures than those commonly employed on hard tissues [5].

The tissue/biological material we focused our attention on were cornea/sclera and cartilage. In this study we report 12 cases where the biological material examined was "unusual": in 6 of them we analyzed corneal/scleral swabs and in 6 we carried out STR analysis on cartilages.

This approach could be considered a good strategy when collecting

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Table 1
Summary of the samples collected and of the obtained results.

Case	PMI	Sample Type	Macroscopic appearance	DNA Quantification	PCR Results
1	7 years	Corneal Swab	Pre-skeletonization/putrefaction	1 ng/ul	+ + +
2	6 years	Corneal Swab	Leathery	1,26 ng/ul	+ +
3	1.5 years	Corneal Swab	Leathery	1,99 ng/ul	+ + +
4	26 years	Corneal Swab	Leathery/ pre-skeletonization	0,63 ng/ul	+ + +
5	8 years	Corneal Swab	Leathery/ pre-skeletonization	219 ng/ul	+ + +
6	9 months	Corneal Swab	Mummification	234 ng/ul	+ + +
7	7 months	Epiglottic cartilage	n.a.	2 ng/ul	+ + +
8	n.a.	Pelvis articular cartilage fragment	Charred	45 ng/ul	+ + +
9	n.a.	Acetabular fossa cartilage	Skeletonized	0,0163 ng/ul	+ + +
10	n.a.	Cartilage from the right ankle and acetabulum	Saponification/ partial skeletonization	0,0432 ng/ul ankle// 0544 ng/ul acetabulum	+ + +
11	n.a.	Intervertebral cartilage of the last thoracic vertebra	Charred	n.a.	+ + +
12	n.a.	Sternum cartilage fragment	Putrefaction	7 pg/ul	+ + +

n.a. = not available.
+ + + = complete profile.
+ + = partial profile (loss of HMW alleles only).
+ = incomplete profile, not suitable for comparison.

cadaveric tissues in case of exhumation, i.e. the cases when we focused our attention on, and in all cases when such “unusual” tissues are available.

2. Materials and methods

Eye swabs and cartilages were taken from 12 cadavers. General information pertaining to the cases examined (PMI, sample type, macroscopic appearance, etc.) are reported in Table 1.

The samples were analyzed in different laboratories with different techniques, in different periods of time.A complete list of the techniques employed is available upon request.

In every case, at least one different tissue was collected and analyzed as double control.

3. Results

The analyses performed have shown good results both quantitatively and qualitatively. The results are reported in Table 1. Quantification data and electropherograms are available upon request.

4. Discussion

The widely recognized improvement of forensic DNA techniques has considerably increased the variety of cases subjected to forensic analyses over the last 2 decades.

The frequency with which lawyers and judges demand DNA tests to establish family relationships have been increasing, even when the proband is deceased, thus presenting new challenges to the analysts.

The exhumation of a corpse presents several complex procedures of sampling, collection, transportation, storage, and analysis of the collected evidence. In these cases, the strategies of sample/tissue collection are largely determined by the appearance of the exhumed corpse, as no other means for valuing the state of preservation of DNA within the tissues is feasible.

It is also largely known that soft tissues degrade more quickly and more severely than hard tissues, like nails, teeth, and bones but the choice between soft or hard tissues is often based only upon the collector’s opinion.

Another point to consider is that it is not unlikely that collector and analyst are not the same people, usually because of the legal nature of a corpse which can be treated only by authorized people. This leads to the setting up of “conservative” procedures aimed at collecting hard tissues rather than soft tissues in order to avoid failures of the analyses. Soft tissues are therefore largely unused when the corpses show signs of moderate/severe degradation.

We have here described several cases where the strategy of sample collection has been widened to specific sampling procedures and tissues, despite their appearance suggested their uselessness due to severe DNA degradation.

The tissues analyzed were cartilage and cornea/sclera. In this last case, simple corneal/scleral swabs were performed.

While cartilage and auditory ossicles have already been described as a source of DNA [6,7] we have not found any reference to eye/corneal/scleral swabs in the scientific literature.

The good results obtained, both from cartilage and corneal/scleral swabs can be explained by the dryer condition of these samples, i.e. their lower content in water which is known to be the basis for DNA hydrolysis [8].

5. Conclusion

These results have prompted us to reconsider the collection strategies of biological tissues during the exhumation procedures. In case of severely degraded corpses, it could be good practice to perform specific samples from the areas of the body which appear to be dryers than others, like cornea/sclera or cartilages.

This does not mean that common “reliable” tissues in terms of DNA profile outcome, like bones, teeth, and nails should not be sampled, but the ease of DNA extraction from corneal/scleral swabs and/or cartilage makes this analysis the first choice prior to long, tedious and expensive DNA extraction procedures from bones and teeth.

The need for several steps with decalcification, EDTA removal, washings, concentration, etc. makes the DNA extraction procedure from bones and teeth much more prone to contamination [5] than the simpler DNA extraction procedure from corneal/scleral swabs.

Therefore, other tissues, even in small, isolated areas, may be useful to be subjected to DNA extraction, despite more research in this area is necessary.

All of this in the light of a higher level of simplification of the DNA extraction procedures in "complex" cases like those arising from exhumed bodies.

Declaration of Competing Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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