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DNA extraction and STR profiling from histological slides

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ABSTRACT

Formalin-fixed paraffin-embedded (FFPE) tissue blocks are commonly used in the field of pathology and forensic pathology as a source of histological slides. For postmortem kinship analysis or identification, DNA can be extracted from blocks with specialized kits. However, when an STR profile should be generated from single microscope slides, the removal of the coverslip and the limited sample size poses unique challenges. We aimed to test the effectivity of agitated xylene incubation to dissolve the mounting material to facilitate the coverslip removal. DNA extraction tests were performed on 5- to 7-year-old histological slides. Xylol was used to dissolve the mounting medium to facilitate cover slide removal, one set of samples was shaken during incubation, and the other set was left still. It was found that shaking the sample while bathed in xylol decreased the incubation time from three days to two days. Agitation not just reduced the processing time but increased the quality of acquired STR profiles: on average 30% more alleles were detected from the shaken samples compared to the still bathed ones.

1. Introduction

Formalin-fixed paraffin-embedded (FFPE) tissue blocks are commonly used in the field of pathology and forensic pathology as a source of histological slides.

From time to time, the need of providing an STR profile from an FFPE block arises, usually due to postmortem identification or paternity cases. While both formalin and xylol (used for removing the paraffin) are well known to decrease DNA integrity [1,2], DNA extraction from FFPE blocks is part of the routine investigations, its protocols are well established, there are kits optimized for this purpose. In Hungary, pathology and forensic medicine departments are required by the law to archive tissue blocks for 25 years.

However, two of our cases of suspected sample mix-up included only microscope slides. We were asked to provide an STR profile from these samples for comparison. A literature search highlighted that the methodological questions are far less addressed than FFPE blocks. The limited amount of tissue, the mounting medium, and the need to remove the cover slide all pose a challenge on their own.

The removal of the coverslip necessitates long xylene incubation to dissolve the mounting material. The study aimed to test if shaking during incubation decreases the duration and might increase the DNA quality.

2. Material studied, methods, techniques

For testing, three pairs of 5–7-years-old microscope slides were used. The three pairs originated from three individuals, contained the same tissue types (lung and liver) and were hematoxylin-eosin stained. All the coverslips were mounted with BioMount medium (BioGnost, Zagreb, Croatia).

The pairs of slides were randomly assigned to one group and were placed into a bottle of xylene bath. The bottles were closed airtight. One of the bottles was shaken at 175 rpm on a Heidolph Unimax 1000 (Heidolph, Schwabach, Germany) platform shaker, the other was left still. Incubation was performed at room temperature. Every twelve hours, we tried to pry off the cover slip with a medical needle under a chemical hood.

After the removal of the cover glass, tissue pieces were scraped off from the microscope slides with a sterile scalpel. DNA was extracted with QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations.

DNA concentration was measured with Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, USA), then amplified by Investigator ESSplex SE QS Kit (Qiagen). Alleles were separated and detected with ABI PRISM® 310 Genetic Analyzer (Thermo Fisher Scientific) and GeneMapper ID v3.2.1 (ThermoFisher Scientific).

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Simulated identification was calculated with Familias v3.3. Allele dropout was considered in non-heterozygous markers (probability: 0.1). Likelihood-ratio (LR) was calculated as full child vs. unrelated.

3. Results

Agitation in xylene decreased the time needed for coverslip removal from 3 days to 2 days.

The measured DNA concentration varied between the sample pairs (Table 1), but there was no significant difference between agitated and static samples (related samples Wilcoxon signed-rank test, $p = 0.593$).

Amplification and detection of the samples were performed in two separate reactions. Alleles were registered only if they were detected in two separate reactions.

Table 1 shows that the number of recovered alleles was higher in the agitated samples compared to the ones with static incubation.

To test, how useful these elevated allele numbers would be in a real-life scenario, we performed kinship analyses with simulated family members, paired with the recovered profiles. The calculated LR was greater by 3–6 orders of magnitude in the agitated sample cases (Table 1).

4. Discussion

This pilot study clearly showed that shaking incubation decreased the number of days necessary for cover slide removal. This limits the time the DNA is in contact with xylene, resulting in less degraded DNA. The fact supports this, that the total amount of DNA recovered from the slide pairs was about the same, and the number of detected alleles increased in the agitated samples.

The simulated kinship analysis models a personal identification case. In two of the three cases, the samples with static incubation resulted likelihood ratios below 10,000. In contrast to that, the agitated samples yielded 13 alleles. The repeated kinship calculations resulted likelihood ratios in the range of millions to hundred millions.

5. Conclusion

Our experiments show that a simple change (shaking) in the protocol can increase the number of recovered alleles and increase LR by up to six orders of magnitude.

Table 1
Results of the three test cases are shown in separate rows. Two microscope slides were chosen in each case and were incubated in xylene for cover slip removal. One sample was shaken on a rotary platform (agitated), the other sample was left in still xylene (static).

	DNA concentration (ng/µl)		Recovered alleles		LR (child vs. unrelated)	
	Static	Agitated	Static	Agitated	Static	Agitated
Test case 1	0.228	0.722	7	13	505	7,025,085
Test case 2	1.74	1.48	8	13	202	499,626,198
Test case 3	4.92	5.12	11	14	61,440	42,445,659

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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