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Examination of pretreatment methods for DNA extraction from nails

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ABSTRACT

In the conventional method of DNA extraction from nails, it takes approximately half a day to dissolve the nails. In this study, we examined whether using the HOrizontal Nail MAshing (HONMA) method, in which pressure is applied to the nail to crush it flat and increase its surface area, would improve DNA extraction efficiency. Fingernails (5 mg) provided by ten volunteers were used as samples. Nail pieces (1–3 pieces), shredded with nail clippers, were thinly stretched by applying 2 t of pressure to each piece using a hydraulic press. DNA was extracted by incubation at 56 °C for 10 min and 1 h during proteolysis. DNA yield from the nails pretreated using the HONMA method increased by 0.20–7.10 times compared with that from unprocessed nails. In particular, 10-min incubation using the HONMA method resulted in an average 2.05-fold increase in DNA yield compared with that under overnight incubation. However, the impact of using the HONMA method varied widely among individuals, and the amount of extracted DNA decreased in some cases, suggesting that the yield may differ depending on the nail quality.

1. Introduction

In DNA analysis for personal identification of unidentified remains, nails are often used as samples when the body decomposes and degenerates. It has been reported that full DNA profiles can be obtained even from the nails of cadavers that have been dead for more than six months [1]. However, in the conventional method of DNA extraction from nails, it takes approximately half to a whole day to dissolve the nails, and larger quantity of DNA cannot be obtained compared to that from blood samples. Efficient DNA extraction from nails in a short time is challenging and has been investigated in previous studies using various extraction reagents [1–3]. In this study, we examined whether processing the shape of the nail used for extraction would improve DNA extraction efficiency. Specifically, the HOrizontal Nail MAshing (HONMA) method, in which pressure is applied to the nail to crush it flat and increase its surface area, was used and compared with conventional extraction methods.

2. Materials and methods

2.1. Sample preparation

Fingernails (5 mg) provided by ten volunteers were used as samples. Nail pieces (1–3 pieces), shredded with nail clippers, were thinly stretched by applying 2 t of pressure to each piece using a hydraulic press (NEC, Tokyo, Japan) (Fig. 1). This project was approved by the Ethical Committee for Human Genome Research at the Kitasato University School of Medicine.

2.2. DNA extraction

DNA was extracted using the QIAamp DNA Investigator Kit (Qiagen, Hilden, Germany) and incubated at 56 °C for 10 min and 1 h during proteolysis. As a control, DNA was extracted from unprocessed nails and incubated under the same conditions. Overnight (18 h) incubation, as recommended by the manufacturer, was also performed. The elution volume was 50 μL.

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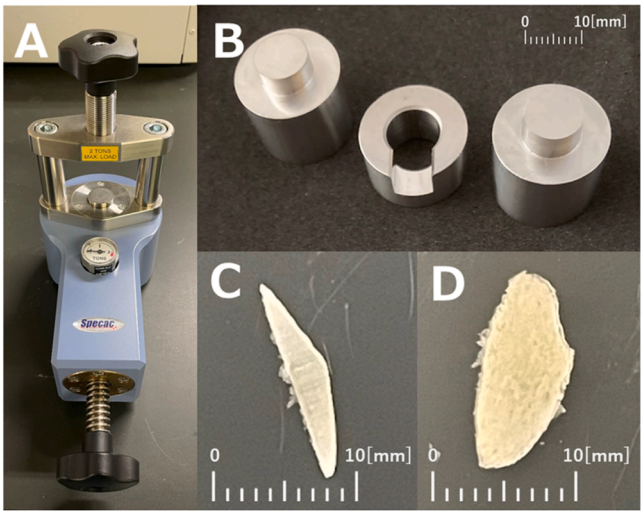

Fig. 1. Instrument used in the horizontal nail mashing (HONMA) method and processed nail. A, Hydraulic press; B, Jig used for crushing nail pieces; C, Pre-processed nail piece; D, Stretched nail piece using the HONMA method.

2.3. Evaluation of DNA quantity and quality

The extracted DNA was quantified using the Quantifiler Human DNA Quantification Kit (Thermo Fisher Scientific, Foster City, CA, USA) and StepOne Real-Time PCR System (Thermo Fisher Scientific).

Short tandem repeat (STR) analysis was performed using the GlobalFiler PCR Amplification Kit (Thermo Fisher Scientific). PCR products were separated using 3500 Genetic Analyzer (Thermo Fisher Scientific) and typed using GeneMapper *ID-X* software v1.4, with the threshold set to 175 RFU. The number of alleles detected in each sample was compared with the total number of alleles in each volunteer, and the detection rate was calculated.

3. Results and discussion

DNA yield from the nails pretreated using the HONMA method was increased by 0.20–7.10 times (average: 1.92 times) compared with that from unprocessed nails under the same incubation conditions. In particular, the 10-min incubation condition using the HONMA method resulted in an average 2.73-fold increase in DNA yield compared with that of unprocessed nails under the same conditions and an average 2.05-fold increase compared with that from overnight incubation. Quantification of DNA under each extraction condition is shown in Fig. 2. No significant differences in DNA yield obtained using the HONMA method and unprocessed nails were observed (Wilcoxon signed rank sum test). However, DNA yield per mg of nail obtained using the HONMA method was more than 4.8 ng/mg, which is more than twice the lower limit of 1.7 ng/mg of DNA yield obtained from unprocessed nails under each incubation condition.

Using the HONMA method, allele detection rate of GlobalFiler under each extraction condition ranged from 86% to 100%, whereas it was from 85% to 100% for unprocessed nails. Allele dropout was observed in some samples, and nonspecific peaks were detected in most samples. These two samples improved allele detection rate in HONMA-10 min. In contrast, the other two samples showed lower detection rates than that of unprocessed nails.

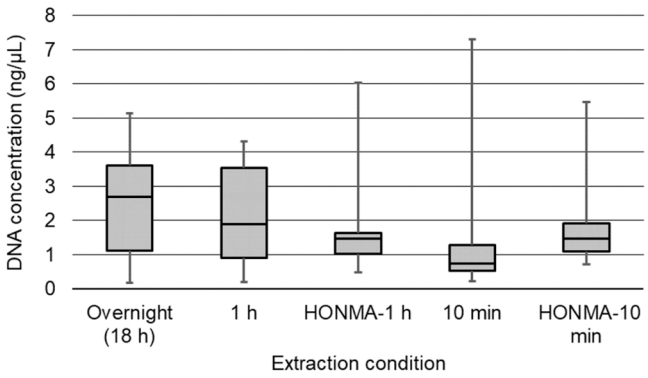

Fig. 2. DNA yield under each extraction condition. Elution volume of 50 μL was used for DNA extraction from 5 mg of fingernail.

The impact of using the HONMA method varied widely among individuals, and the amount of extracted DNA decreased in some cases, suggesting that the yield may differ depending on the nail quality.

4. Conclusion

The HONMA method was used to examine whether the DNA extraction efficiency could be improved by crushing the nails flat. According to the results, particularly under 10-min incubation condition, DNA yield obtained using the HONMA method was an average of 2.05 times the amount of DNA obtained using conventional overnight extraction, and the STR allele detection rate was comparable. Therefore, this method is expected to be applied as a shorter extraction method for nail samples. We propose to apply the HONMA method to the nails of cadavers.

Role of the funding source

The planning of this study and design of the experiments were done in collaboration with NEC Corporation.

Conflict of interest statement

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