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Research article

Optimization of DNA recovery from toothbrushes

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Abstract

In human identification, the victim's toothbrush is an invaluable personal item as the deposited cellular material contains DNA from which a reference profile can be produced. The profile obtained then allows direct comparison to be made with the profile from the unidentified body. This study was undertaken to determine the minimum number of bristle bundles that would generate a complete DNA profile. The minimum period of usage for a toothbrush to retain enough cells for genotyping was also investigated. We also tested two commonly used DNA extraction methods: QIAamp® DNA Mini Kit and Chelex® 100 to explore the efficiency of these protocols in recovering DNA from toothbrushes. In this experiment, volunteers brushed their teeth for 1, 7, 14, or 30 days. DNA was extracted from 5 and 10 bundles of bristles cut from the collected toothbrushes. The amount of DNA recovered was quantified by quantitative real-time PCR, and DNA genotyping was performed for each sample. Data revealed that QIAamp® DNA Mini Kit performed better at yielding DNA in terms of purity, quantity, and quality than Chelex® 100. It was also found that, with a suitable method of recovery, DNA samples from five bundles of bristles from all of the toothbrushes generated complete profiles. Based on the experimental results, a general guideline concerning the appropriate extraction method and the quantity of the starting material for the analysis of DNA from toothbrushes could be suggested.

Keywords: Toothbrush; DNA extraction; Quantitative real-time PCR; DNA profiling

1. Introduction

Toothbrushes are an often selected source of reference DNA sample in victim identification cases [1,2]. Although profiling DNA from toothbrushes is routinely performed, there have been reports of failure to generate results in some cases in Thailand. One cause could be attributed to the moisture in bathrooms together with the tropical temperature and humidity, which promotes growth of bacteria and thus accelerates DNA degradation [3]. Furthermore, although the common practice of using all of the bristles improves the chance of collecting enough intact genetic material, it may also increase the concentration of PCR inhibitors found in toothpaste residue, which compromises the quality of the resulting DNA profile.

In this experiment, we compared the quantity and quality of DNA recovered with two different extraction methods: Chelex® 100 (Bio-Rad) and QIAamp® DNA Mini Kit (QIAGEN), from toothbrushes which had been used for varying periods of time. To minimise the effect of co-extracted PCR inhibitors, we investigated the minimum number of bristle bundles that would enable complete DNA profiles to be generated under laboratory's standard PCR and profiling conditions.

2. Materials and methods

Four volunteers brushed their teeth with the given toothbrushes for 1, 7, 14, or 30 days. For each toothbrush, two sets of 5 and 10 bundles of bristles were collected (Fig. 1). One set was subjected to extraction using Chelex® 100 [4], and the other set was subjected to QIAamp® DNA Mini Kit extraction according to the protocol for oral swabs. DNA concentration was estimated using Quantifiler™ Human DNA Quantification Kit (Applied Biosystems) and ABI PRISM® 7000 Sequence Detection System. After quantitation, samples with DNA concentration of approximately 0.025 ng/μL or above were selected for amplification with AmpFlSTR® Identifiler® PCR Amplification Kit (Applied Biosystems) using a GeneAmp® PCR System 9700 (Applied Biosystems). This is to ensure that there is at least 0.5 ng of template DNA in the total reaction volume of 50 μL. STRs amplification was performed using GeneAmp® PCR System 9700 (Applied Biosystems) accord-

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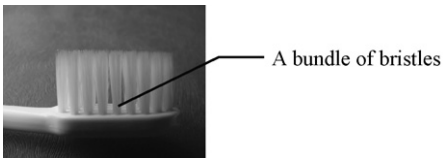

Fig. 1. Illustration of a bundle of toothbrush bristles.

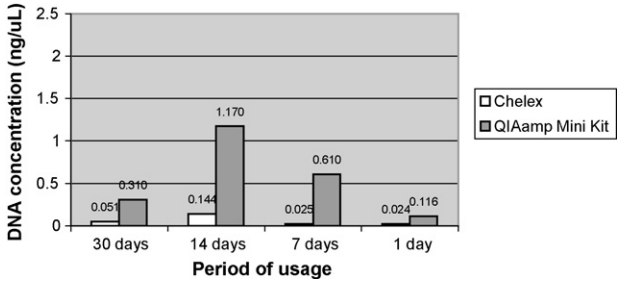

Fig. 2. Comparison of the DNA yield from two extraction methods using five bristle bundles as the starting material.

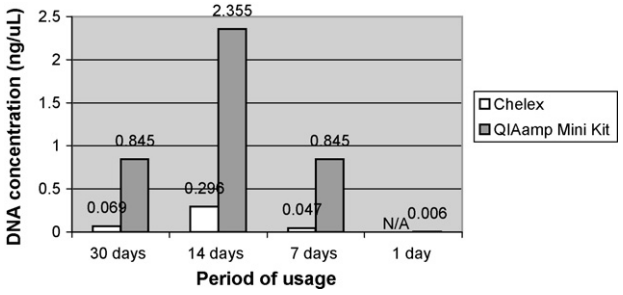

Fig. 3. Comparison of the DNA yield from two extraction methods using 10 bristle bundles as the starting material.

ing to the Kit's protocol. Fragment analysis was carried out using ABI PRISM® 3100 Genetic Analyzer and GeneMapper™ ID Software (Applied Biosystems).

3. Results

Data from the quantitative real-time PCR (Fig. 2) showed that when using five bundles of bristles, QIAamp® DNA Mini Kit yielded larger amounts of DNA than Chelex® 100 for all periods of usage by at least threefold. When 10 bundles of bristles were used, the difference was increased to at least fivefold, except for the 1 day sample where the amount of DNA was undetectable (Fig. 3). STRs analysis revealed that complete profiles were obtained from all genotyped samples recovered from five bundles using Mini Kit (Fig. 4). When Chelex® was used, an almost complete profile could be generated only from the 14-day toothbrush, with one allele dropped out. DNA obtained from 10 bristle bundles of the 30- and 14-day toothbrushes with Mini Kit were the only two periods from which complete profiles could be generated (Fig. 5), while no full profile could be produced from any of the samples extracted with Chelex®.

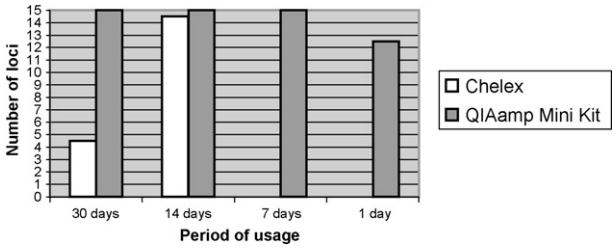

Fig. 4. Comparison of the number of detected Identifiler® STR loci in samples extracted from five bristle bundles using the two extraction methods.

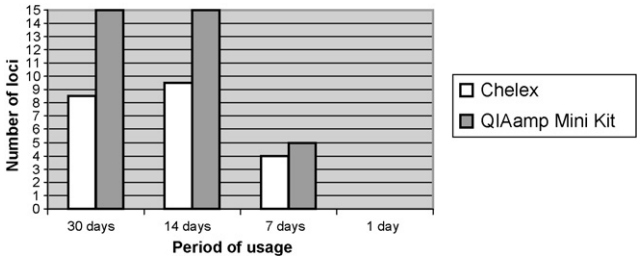

Fig. 5. Comparison of the number of detected Identifiler® STR loci in samplesextracted from 10 bristle bundles using the two extraction methods.

4. Conclusions

Results indicated that QIAamp® DNA Mini Kit performed better than Chelex® 100 at recovering genomic DNA with enough quantity and quality to generate complete DNA profiles. The same outcome is reached in most of the periods of usage examined. Comparison of the number of detectable STR loci among the samples suggested that five bristle bundles is the suitable amount of starting material for DNA extraction from toothbrushes. However, further studies are required to confirm that these findings are reproducible in larger sample sizes, and whether the person-to-person variation has any significant effect before a definite guideline can be established.

Conflict of interest

None.

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