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Toenails as an alternative source material for the extraction of DNA from decomposed human remains

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ABSTRACT

The DNA identification of decomposed human remains for coronial investigations at the Victorian Institute of Forensic Medicine routinely requires the retrieval and processing of a bone sample obtained from the deceased. Bone is a difficult sample type to work with as it requires surgical removal from the deceased, refrigerated storage, and additional processing steps prior to DNA analysis in comparison to other samples types such as buccal swabs or blood stains. In an attempt to overcome the issues posed by bone, a DNA extraction method utilising toenails as an alternate source material was optimised and trialled.

Two DNA extraction methods were optimised for digestion of toenail material, with the method utilising the QIAGEN DNA Investigator Kit selected for a casework trial. Single source DNA profiles, matching those of the conventional samples taken, were obtained for toenail samples collected from 28 of 30 coronial cases available for this study. Of these, 26 toenail samples produced full profiles. Although the overall DNA profile quality from the toenails was less than that of the conventional sample, the profiles from toenails met the reporting requirements for identification. Based on the results obtained, the Victorian Institute of Forensic Medicine will be implementing toenails as the primary sample type for collection from decomposed remains when blood is not a suitable sample type.

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1. Introduction

In the State of Victoria, Australia, deceased individuals (not suitable for visual identification) are typically identified by comparison of the DNA profile obtained from a sample of the deceased to the DNA profile of a reference sample from a relative or a known sample of the deceased; a process performed by the Victorian Institute of Forensic Medicine (VIFM). The sample routinely obtained for identification in decomposed cases is bone, as blood (suitable for DNA analysis) is rarely obtained from decomposed remains. Bone typically provides a good yield of adequate quality DNA due to its hard structure protecting the DNA from degradation [1] leading to Short Tandem Repeat (STR) DNA profiles suitable for identification purposes.

Despite the success of bone as a primary sample type for decomposed cases, its retrieval and processing for DNA identifications has some associated issues including; the invasiveness of the surgical procedure to remove the bone from the deceased; the occupational health and safety (OH&S) risks to staff when using saws to retrieve and sample the bone; the time taken to prepare and sample the bone; and the requirement for refrigerated storage of the sample.

Many of the forensic studies analysing nail material focus on identifying perpetrators of crimes from fingernail scrapings of the victims (see [2–6]). There are few studies to date that describe the extraction of DNA from nail material from decomposed remains for identification purposes (some examples are displayed by [7–9]). Toenails were selected for this study as they are less exposed to exogenous DNA sources due to feet often being protected by shoes or socks. This idea that toenails may display lower numbers of mixed profiles when compared to fingernails has been suggested previously [7].

The theory underpinning the use of nails for DNA extraction is that, analogous to bone, they are composed of a hard biological material which is resistant to environmental damage and the effects of decomposition [10,11]. In comparison with bone, toenails can be collected easily and non-invasively which results in fewer

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OH&S risks [7,12]. Nail material may also require less time to process to obtain a DNA profile [7] leading to a quicker identification of the body which in turn provides faster answers to distressed families waiting for the identification results. In the circumstances of a mass fatality event, nails also have the benefit of being able to be collected by persons with minimal training in sample collection and take up minimal storage space without the requirement for refrigeration [7,13].

In an effort to overcome the issues associated with bone samples for decomposed cases, a study was commenced to develop, validate and implement a method using toenails as the source material for DNA extraction. Described in this study is the optimisation of two DNA extraction methods for toenail material – one organic and one utilising a silica based column purification. The application of the silica based method for the extraction of DNA from toenail samples for 30 decomposed coronial cases, and the comparison to the conventional sample taken, is also described.

2. Materials and methods

2.1. Extraction protocol selection

Two extraction protocols optimised by the Molecular Biology Laboratory (MBL) at the VIFM for hair samples were selected as the basis for optimisation using toenails as the source material. One protocol utilised the Qiagen DNA Investigator Kit and the other comprised of an in-house prepared digestion buffer with phenol chloroform purification and subsequent concentration by filtration.

2.2. Samples

2.2.1. Toenails and buccal swabs from live donors

Consenting donors were asked to provide clippings of their own toenails for use in the study. Buccal swabs were taken as the standard sample from the donors. DNA profiles obtained from the buccal swabs were compared to profiles obtained from the corresponding toenails to confirm validity.

2.2.2. Mortuary samples

In all cases, the toenail sample was collected along with the conventional post mortem sample for that case. The choice of which conventional sample type to take was dependent on the condition of the body (extent of decomposition) with blood being collected in less decomposed cases and bone taken with the increasing level of decomposition.

The toenail sample was collected either by clipping the tip of the nail from the toenails of the deceased or, where the level of decomposition dictated, the removal of the entire nail from the toe. Following collection, samples were transferred to the MBL for DNA analysis.

2.3. DNA extraction method optimisation

The extraction methods used in this study were optimised using nail material donated by live volunteer donors. Profiles obtained from the donor toenails were compared to the donor's corresponding buccal swab sample.

The parameters optimised for each extraction method were: the amount of source toenail material required for extraction; the duration of the digestion of toenail material in buffer (compared 0.5 h, 1 h, 2 h, 4 h, 6 h, and 24 h digestion times); whether a pre-wash step was required before the nails were digested and the duration of the wash time when included (compared 0.5 h, 1 h, 1.5 h, and 2 h wash times).

2.4. DNA extraction methods for toenails

2.4.1. QIAGEN DNA investigator kit method

At least 0.01 g of toenail fragment(s) were weighed in a 1.5 mL tube and 300 μL of Buffer ATL (Qiagen), 20 μL 20 mg/μL Proteinase K (Qiagen) and 20 μL 1 M DTT (Sigma–Aldrich) added. The tube was vortexed for 10 s and incubated with agitation for 0.5 h on a thermomixer (900 rpm) at 56 °C. The manufacturer's protocol 'Isolation of Total DNA from Nail Clippings and Hair' was followed for the rest of the process using a manual extraction method with carrier RNA included. Samples were eluted in 100 μL of Buffer ATE (Qiagen). A pre-wash of the volunteer nail samples was not required for the final developed method.

A number of modifications were applied to the optimised method for the casework toenail sample extractions. The nail was held with forceps while the surfaces of the nail were scraped using a scalpel to remove material such as decomposed flesh and dirt. The nail fragment(s) were rinsed in 1 mL of 70% (v/v) ethanol (VWR International) solution vortexed for 30 s in a 1.5 mL tube. The solution was discarded and the wash repeated using an additional 1 mL of 70% (v/v) ethanol solution. Nail fragment(s) were left to dry for 5 min before being placed in a new 1.5 mL tube for extraction. Toenail samples were extracted following the manufacturer's protocol "Isolation of DNA from forensic casework samples" using a QIAcube for automation (cases 5–30), or manually (cases 1–4) following the 'Isolation of Total DNA from Nail Clippings and Hair' protocol as detailed above.

2.4.2. In-house digestion buffer method

At least 0.01 g of toenail fragment(s) were weighed in a 1.5 mL tube. To wash the nail material, 50 μL of 1 M NaCl (Sigma), 50 μL of 100 mM EDTA (Merck), 10 μL 20% (w/v) SDS (ICN Biomedicals Inc.), 6.25 μL of 2 mg/μL Proteinase K, and 380 μL of dH_2O were added and the sample incubated for 0.5hr with agitation at 56 °C in a thermomixer (900 rpm). The solution was discarded and the nail material rinsed twice with 500 μL of 0.9% (w/v) NaCl, once with 500 μL of 100% (v/v) ethanol, and then with 500 μL of dH_2O discarding the solution after each wash step.

To the washed nails, 400 μL of TEN buffer (10 mM Tris–HCl/pH8.0, 1 mM EDTA, 100 mM NaCl), 50 μL of 20% (w/v) SDS, 40 μL of 20 mg/uL Proteinase K, and 40 μL of 1 M DTT were added, followed by an incubation at 56 °C with agitation using a thermomixer (900 rpm) for 2 h to digest the nail material. Following digestion, an equal volume of ultrapure phenol:chloroform:isomyl alcohol (25:24:1) pH 8.0 (Invitrogen) was added to the tube, shaken and then centrifuged at 13,500rcf for 3 min. The upper phase was removed, added to a new 1.5 mL tube and an equal volume of chloroform added. The tube was mixed and centrifuged at 13,500 rcf for 1 min, this chloroform wash step was performed twice. The resulting upper phase was transferred to an Amicon Ultra 0.5 100 K device and spun at 14,000 rcf for 2 min. The filtrate was discarded and the Amicon washed twice with 480 μL of dH_2O. 100 μL of dH_2O was added to the Amicon which was then inverted into a collection tube and spun for 2 min at 1000 rcf to elute.

2.5. DNA extraction for conventional samples

For the majority of the conventional casework samples (28/30), the samples – bone or bloodstain – were extracted as previously described [14]. For one case (case 29), the shaft of the humerus was sampled. For another case (case 30), the extraction using the femur head failed and so material from the femur shaft was sampled.

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