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TECHNICAL NOTE

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An alternative for the Extraction and Storage of DNA from Insects in Forensic Entomology*

ABSTRACT: An important area of recent research in forensic entomology has been the use of insect DNA to provide identification of insects for fast and accurate estimation of time since death. This requires DNA to be extracted efficiently and in a state suitable for use in molecular procedures, and then stored on a long-term basis. In this study, Whatman FTA™ cards were tested for use with the Calliphoridae (Diptera). In particular, testing examined their ability to effectively extract DNA from specimens, and store and provide DNA template in a suitable condition for amplification using the polymerase chain reaction (PCR). The cards provided DNA that was able to be amplified from a variety of life stages, and thus appears to be of sufficient quality and quantity for use in subsequent procedures. FTA cards therefore appear suitable for use with calliphorids, and provide a new method of extraction that is simple and efficient and allows for storage and transportation without refrigeration, consequently simplifying the handling of DNA in forensic entomological cases.

KEYWORDS: forensic science, forensic entomology, calliphorid, DNA, Whatman FTA

The utility of DNA in forensic entomology has become a major area of research in recent years. Researchers have firmly established the potential to characterise insects of forensic importance to species level on the basis of specific regions of DNA (1–4).

Forensic entomologists most commonly employ DNA based techniques for use with calliphorids, or blowflies, due to their general ubiquity at crime scenes, and their unique ability to be among the first insects to locate a corpse following death (5). This makes them particularly important, as they most frequently begin the predictable insect succession on a corpse, and are useful in estimation of time since death. Generally it is the immature stages that require DNA based identification due to the high level of morphological similarity between species, but adults may also require analysis.

The application of DNA based technologies to any field requires the development and optimisation of protocols suitable for the extraction and long-term storage of samples. An extraction technique should preferably be simple, efficient, provide DNA of sufficient quality and quantity to be utilised in subsequent procedures, and ideally, involve non-hazardous reagents. Storage issues include the long-term viability of the sample, preserved in a state suitable for reanalysis should such a request arise.

Traditionally the extraction methods utilised in this field have included Chelex based techniques and variations on phenol:chloroform extraction (1–3). Samples are subsequently frozen for long-term storage. These techniques provide DNA suitable for use in subsequent procedures, however the hazardous nature of the phenol:chloroform reagents is obviously disadvantageous. Techniques that improve the extraction, storage and handling of DNA, whether through cost, labour or safety are obviously useful alternatives to be considered.

FTA™ cards (Whatman BioSciences) provide an ideal alternative for the extraction and storage of samples. The technique involves application of a sample to the card, and the FTA-treated paper lyses cells within the sample and immobilises the released DNA. The sample dries, and a disc may be punched from the card and prepared through a series of washes to be used directly in the polymerase chain reaction (PCR). Cards may be stored at room temperature.

The cards have been utilised for forensic purposes in the collection and processing of DNA samples (6). Other applications have included use with corals (7), and also DNA extraction from saliva from beetle quid chewers, where inhibitors in the sample created difficulties for subsequent DNA amplification but were eliminated using the FTA™ method (8).

The cards hold several specific advantages for use in this field if proven suitable for use with entomological samples. They provide a fast and simple method of preparing the sample, and reduce chances of contamination as liquid samples are no longer being handled but small discs of paper. Transportation of samples, either between entomologists or simply from one location to another does not require refrigeration, and long-term storage issues such as freezer space and potential desiccation of samples become less pertinent.

This study tested the ability of the cards to produce DNA of sufficiently high quality and quantity to be employed in the polymerase chain reaction (PCR). The main criteria assessed were the overall ability to be amplified, potential for use with a variety of insect life stages, and ability to amplify products of considerable size to ensure samples are not significantly degraded during the extraction process. Species tested in this study were all members

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of the dipteran family Calliphoridae: *Chrysomya megacephala* (Fabricius), *Chrysomya rufifacies* (Macquart), *Lucilia sericata* (Meigen), *Calliphora dubia* (Macquart) and *Cochliomyia hominivorax* Coquerel. Various life stages were employed, and conclusions drawn as to the potential application of FTA™ cards in this field.

Materials and Methods

Insects used in the study were collected from the field and represented a variety of calliphorid species. These species were *Chrysomya megacephala*, *Chrysomya rufifacies*, *Lucilia sericata*, *Calliphora dubia* and *Cochliomyia hominivorax*. Adult specimens were stored in 70% ethanol; larvae and pupae were first subjected to 30 sec in boiling water then stored in 70% ethanol.

Preparation of the insect material for application to the FTA™ cards varied according to the life stage used. Flight muscles of adult flies were removed through a small incision in the right side of the thorax, and the remaining insect kept as a voucher specimen. In the case of pupae, the organism was removed from the casing, and the pupal casing itself retained as a voucher. For larval specimens, both second and third instar larvae were used, generally with the middle third of the insect being selected and the morphologically characteristic anterior and posterior segments retained for any further verification.

Specimens were ground in 100 μL 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA, Fisher Scientific), pH 7.5 using a micropestle in a 2 mL tube until the solution was partially homogenised. Following grinding, the sample was applied to an FTA card and allowed to dry for a minimum of 1 h.

To verify the successful lysis of cells, and binding and extraction of DNA, samples were prepared for amplification using PCR. Discs of both 1.2 mm and 2.0 mm diameter were prepared to determine the optimal sized disc for use with calliphorid samples. The manufacturer's protocol was followed for the extraction from the cards. Two washes were found to be necessary using the FTA™ Purification reagent (Whatman BioSciences), and two washes using 1X TE buffer.

Larval, pupal and adult samples were all prepared, and tested using PCR. To confirm the quality, and therefore low degradation of the sample during preparation, PCR reactions were performed to amplify fragments of varying sizes. The fragments amplified all lie within the cytochrome oxidase I (COI) encoding region of mitochondrial DNA (mtDNA). These were approximately 320, 650 and 1270 base pairs in size and are listed in Table 1.

PCR reaction mixes consisted of: 1X PCR buffer containing 1.5 mM $MgCl_2$ (Fisher Scientific), 200 μM dNTPs (Applied Biosystems), 25 pM each primer, 1 unit Taq polymerase (Fisher Scientific) and water added to a total volume of 50 μL. Cycling conditions for the 320 bp fragment were taken from Harvey et al. (4), and for the two other primer pairs from Harvey et al. (1).

Electrophoresis of PCR products was performed using 1.5% agarose gels stained with ethidium bromide.

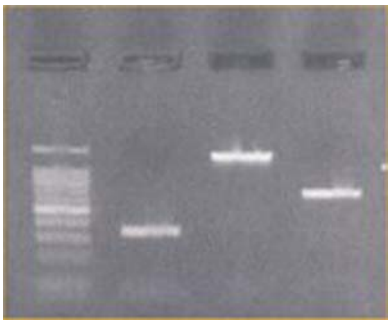

FIG. 1—*Picture of 1.5% agarose gel showing fragments of 320, 1270 and 650 base pairs successfully amplified using discs prepared from FTA cards.*

Results

Discs were prepared from multiple individuals from several calliphorid species, and subjected to PCR. All samples were successfully amplified, including larval, pupal and adult samples. PCR products were clear, intense and of the expected product size when viewed on agarose gels.

Several samples were tested to determine the optimal disc size for use in reactions. Discs were trialled in concurrent reactions and results verified through repetition, and amplified bands were greater in intensity when template was provided in the smaller (1.2 mm) disc size.

To ensure the overall quality of the template, and thus minimal degradation of the sample during the lysis, binding and washing processes, PCR reactions amplifying various sized products were conducted. Figure 1 displays the resulting products, with 320, 650 and 1270 base pairs successfully amplified from a variety of species and life stages.

Discussion

This study shows the potential for use of FTA™ cards for forensic entomological applications. The protocols used prepared DNA in a suitable condition for use in subsequent molecular procedures.

The ability to amplify samples from a variety of calliphorid life stages is desirable, as any stage may be collected from a scene and require analysis in its current stage. Immature and adult stages vary markedly in morphology, and thus the physical and chemical composition of the stages may also vary. The cards successfully lysed cells from both immatures and adult flight muscles, and provided DNA in a state suitable for use in PCR. Any potential inhibiting compounds were therefore successfully removed.

The optimal disc size suggested in the Whatman protocol was the larger, 2.0 mm disc. The PCR products produced with the smaller disc were generally more intense in this study, perhaps indicating that sufficient DNA is lysed from the samples for use of a smaller disc. The larger discs may be providing considerably more template

TABLE 1—*Primer pairs used to amplify the three fragments tested in this study.*

Primer Pair	Fragment Size	Primer & Sequence	Source
1	320 bp	C1-J-2495 (5′ CAGCTACTTTATGAGCTTTAGG 3′) C1-N-2800 (5′ CATTTCAAGCTGTGTAAGCATC 3′)	1, 2, 4
2	650 bp	UEA7 (5′ TACAGTTGGAATAGACGTTGATAC 3′) TL2-N-3014 (5′ TCCAATGCACTAATCTGCCATATTA 3′)	9, 10
3	1270 bp	C1-J-1718 (5′ GGAGGATTTGGAAATTGATTAGTTCC 3′) TL2-N-3014 (as above)	1, 10

to the reactions, and thus reducing the efficiency of the PCR system.

The ability to amplify a fragment of up to 1270 base pairs from the template prepared using the cards indicates that the DNA is of sufficient quality to be used in molecular procedures. Further testing would be required to confirm amplification of larger fragments, but it appears that the DNA being extracted is of considerable quality and should not prove difficult to manipulate in other procedures.

Obvious advantages of these cards include storage issues, as samples are easily stored in a stable form on a piece of card, eliminating the need for freezing of samples. This also reduced the chances of both contamination and desiccation of a sample. Samples are also transportable at room temperature, and most infectious agents are considered to be deactivated on contact with the card, thus removing potential biohazards (6).

Whatman FTA^{TM} cards therefore provide DNA of a suitable quality and quantity for use in forensic entomology, and hold several advantages over traditional methods of extraction and storage used in this field.

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References

1. Harvey ML, Mansell MW, Villet MH, Dadour IR. Molecular identification of some forensically important Calliphoridae (Diptera) of southern Africa and Australia. Med Vet Entomol 2003;17:363–9. [PubMed]
2. F. A. H. Sperling, Anderson GS, Hickey DA. A DNA-based approach to the identification of insect species used for postmortem interval estimation, J Forensic Sci 1994;(39):418–27. [PubMed]
3. Benecke M. Random amplified polymorphic DNA (RAPD) typing of necrophagous insects (diptera, coleoptera) in criminal forensic studies: validation and use in practice. Forensic Sci Int 1998;98:157–68. [PubMed]
4. Harvey ML, Dadour IR, Gaudieri S. Mitochondrial DNA cytochrome oxidase I gene: potential for distinction between immature stages of some forensically important fly species (Diptera) in Western Australia. Forensic Sci Int 2003;131–4. [PubMed]
5. Byrd JH, Castner JL. Insects of forensic importance. In: Byrd, JH, Castner, editors. Forensic entomology: The Utility of Arthropods in Legal Investigations. USA: CRC Press, 2001;43–79.
6. http://whatman.forensic.e-symposium.com/expert/
7. Crabbe JMC. A novel method for the transport and analysis of genetic material from polyps and zooxanthellae of scleratinian corals. J Biochem and Biophys Methods 2003;57:171–6.
8. Salvador JM, De Ungria MCA. Isolation of DNA from saliva of beetle quid chewers using treated cards. J Forensic Sci 2003 July;48:1–4.
9. Zhang D-X, Hewitt GM. Assessment of the universality and utility of a set of conserved mitochondrial COI primers in insects. Insect Mol Biol 1997;6(2):143–50. [PubMed]
10. Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. Evolution, weighting, and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain-reaction primers. Ann Entomol Soc Am 1994;87:651–701.

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