Direct PCR using microFLOQ® direct swabs with a modified QIAGEN Investigator 24plex GO! protocol from decomposing human remains for DVI applications

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INTRODUCTION

The identification of human remains after any mass fatality event is an essential process, but many challenges are often encountered. Large natural disasters can destroy infrastructure [1], and heat from tropical climates accelerates decomposition of human remains, making it difficult to maintain sample integrity [2, 3]. Therefore, the speed of collection and ease of storage are primary considerations for disaster victim identification (DVI) efforts. Typical laboratory storage methods like refrigeration or freezing may not be available and therefore it is necessary to identify reliable, cost-efficient methods that are robust enough to tolerate environmental conditions in which DVI scenarios may occur.

Collection of biological material from decomposing bodies using swabs may prove easy, efficient, and amenable to storage in harsh conditions. microFLOQ® direct (Copan Italia, Bresica, Italy) swabs have been identified as a potential alternative for more rapid collection and processing of DNA in forensic and DVI situations [4]. Direct PCR processing with microFLOQ® swabs have been compared to traditional methods, and were found to have similar or superior results for DNA sources like bloodstains and common casework samples [5, 6]. The microFLOQ® fibers contain a lysing agent to aid in direct PCR and a desiccation agent in the cap for rapid drying and prolonged storage. The potential storage capability of swabs is useful in DVI scenarios as remains can be swabbed and retained at room temperature (RT) until processing.

Direct amplification is a strategy commonly used with reference samples to reduce time and cost by eliminating extraction and quantification steps of DNA processing. Direct amplification kits are tolerant of higher DNA inputs and inhibitors [7, 8], and direct PCR is more efficient at utilizing the DNA collected than traditional extraction-based processing where approximately 50-90 percent of DNA can be lost during extraction [9]. The inhibitors present in DVI-type samples may pose a challenge for direct amplification. Because of the potential inhibition challenges, both the Investigator 24Plex GO! Direct Amplification Kit (QIAGEN, Hilden, Germany) and the Investigator 24Plex QS Kit (QIAGEN) were chosen for their ability to monitor inhibition during STR amplification with their internal quality sensors.

In this pilot study, Genetics 4N6FLOQSwabs® and microFLOQ® direct swabs (Copan Italia) were used to collect DNA from red muscle via an incision in the arm or leg of a decomposing human cadaver. Traditional DNA processing with the Genetics swabs was compared to a direct amplification strategy using the microFLOQ® swab coupled with the Investigator 24plex QS GO! Kit. All swabs were tested for their ability to collect and store DNA at RT. Direct amplification and traditional methods were compared to identify the most effective approach for DVI sample collection and processing.

MATERIALS & METHODS

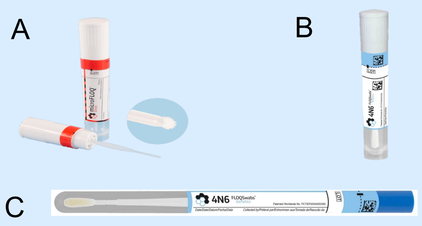

Figure 1: (A) microFLOQ® direct swab, (B) Short Genetics 4N6FLOQSwab®, (C) Genetics Long 4N6FLOQSwab®; images courtesy of www.copangroup.com.

Figure 2: NAO spin basket used during lysis; image courtesy of www.copangroup.com.

Collection & Storage: Muscle and swab samples were collected at the Applied Anatomical Research Center (AARC) at Sam Houston State University in Huntsville, TX, USA during the month of November 2018 (temperatures were highly variable; ranged from 28°C to 0°C). Three types of sample were taken: swabs, tissue biopsy punches, and archival muscle tissue. Three swab types (microFLOQ® direct swabs (**Fig 1A**), Short Genetics 4N6FLOQSwabs® (**Fig 1B**), and Long Genetics 4N6FLOQSwabs® (**Fig 1C**) were collected from the same areas as the tissue samples, with red muscle being swabbed. The swabs were collected on six days (0, 1, 4, 10, 13, 20). Swabs were collected for three storage times (0, 1, 3 months) at RT (25ºC). The three swab types were collected in triplicate for each storage time from both the upper and lower body, totaling 54 swabs (N=18 for each swab) a day. To note, this poster does not show results of Long Genetics swab.

DNA Extraction: Tissue biopsies were extracted using the EZ1 DNA Investigator Kit (QIAGEN). The Genetics 4N6FLOQSwabs® were processed with the EZ1 DNA Investigator Kit following the Forensic "Contact Swab" protocol with the exception of NAO spin baskets (**Fig 2**) (Copan Italia) used during a static lysis.

microFLOQ® pre-processing: 1mL distilled autoclaved water to the microFLOQ® tube and vortexed. Swabs were then broken into PCR strip tubes, 2 μL of 24Plex GO! Lysis buffer was added, and the swabs were lysed at 95°C for 5 minutes prior to amplification.

DNA Quantification: Samples were quantified using the Investigator Quantiplex Pro DNA kit (QIAGEN) on a 7500 Real-Time PCR System (Thermo Fisher Scientific, South San Francisco, CA).

Genotyping: STR amplification was performed on DNA extracts using the Investigator 24plex QS kit as per the manufacturer's protocol [36]. Amplification of the pre-processed microFLOQ® swabs was performed using the Investigator 24Plex GO! Direct Amplification Kit (QIAGEN) with modification to the cycling parameters (doubled two final extension steps). Amplified products were separated and detected on a 3500 Genetic Analyzer (Thermo Fisher Scientific). Data were analyzed on GeneMapper IDX v.1.4 using an analytical threshold of 100 RFU and a stochastic threshold of 200 RFU. Additionally, tertiary analysis was performed with an in house excel workbook.

Subsampling: Subsampling was performed using microFLOQ® swabs to collect and amplify DNA from the Genetics 4N6FLOQSwabs® at 3 months of storage. For this purpose, the microFLOQ® swabs were moistened with 2μL autoclaved water then used to swab vertically and around the tip of the Long Genetics 4N6FLOQSwabs®. The microFLOQ® swabs were dried for one hour and processed as per the methods described in the "microFLOQ® pre-processing" and "Genotyping" sections.

RESULTS & DISCUSSION

- Decomposition progressed to active decay over the 20 days as incisions were made progressively down each limb for sampling (**Fig 3**).
- DNA concentration dropped severely after 13 days of decomposition (**Fig 4**), but full STR profiles were still obtained with biopsy controls and Short Genetics 4N6FLOQSwabs® (**Fig 5**).
- No significant difference was observed in profile completeness across storage times (**Fig 5**).
- Both swab types were able to preserve DNA for up to 3 months when collected by Day 4. However, when microFLOQ® swabs were used to collect biological material from the decomposing cadaver after Day 4, PCR inhibition affected allele recovery (**Fig 5 and 6**).
- Inhibition was confirmed by Quality Sensors (QS). Inhibition was tolerated when higher amounts of DNA in days 1-4 where available for amplification (**Fig 5**).
- By subsampling the Long Genetics 4N6FLOQSwabs® swabs with the microFLOQ® swabs, more complete profiles were observed on Day 10 compared to the microFLOQ® direct samples.
  - A reduction in PCR inhibition was also observed with the subsampling method compared to direct swabbing with the microFLOQ® swabs (**Fig 6 and 7**).

Figure 3: Photos of the upper limb of the cadaver when sampled on (A) Day 0, and (B) Day 20 showing the degree of decomposition over the sampling period.

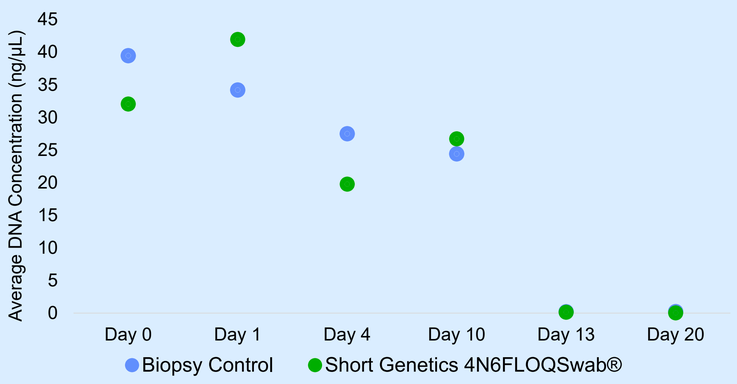

Figure 4: DNA concentration from samples collected using the Short Genetics 4N6FLOQSwab® and biopsy tissue (No storage). Both swab and biopsy data are averages of arm and leg quantities (N=6 for swabs and N=2 for tissue). Error bars were not added for ease of viewing.

Day	Biopsy Control	Short Genetics 4N6FLOQSwab®			microFLOQ® direct			Subsampling		
		Month 0	Month 1	Month 3	Month 0	Month 1	Month 3	Month 0	Month 1	Month 3
0										
1										
4										
10										
13										
20										

100% | 90% - 99% | 75% - 90% | 50% - 75% | 0% - 50%

Figure 5: Heat map of STR completeness (Allele %) comparing traditional and microFLOQ® processing methods. Colors displayed in each box are the average allele completeness of 6 swabs (3 arm and 3 leg).

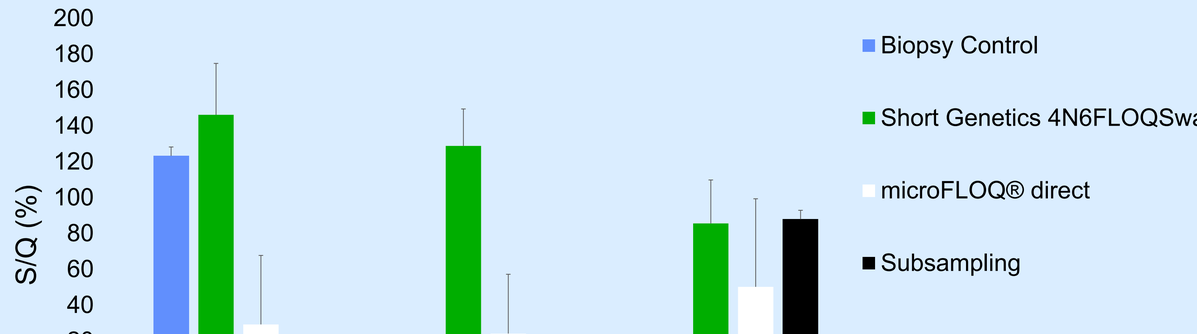

Figure 6: Average QS scores across 3 months for all swab types and biopsy tissue. Biopsy, N=12; Short Genetics 4N6FLOQSwab®, microFLOQ® direct, Subsampling, N=36. Error bars represent standard deviation. Red dotted line indicates 20% Q/S ratio threshold used as an indication of likely PCR inhibition during amplification.

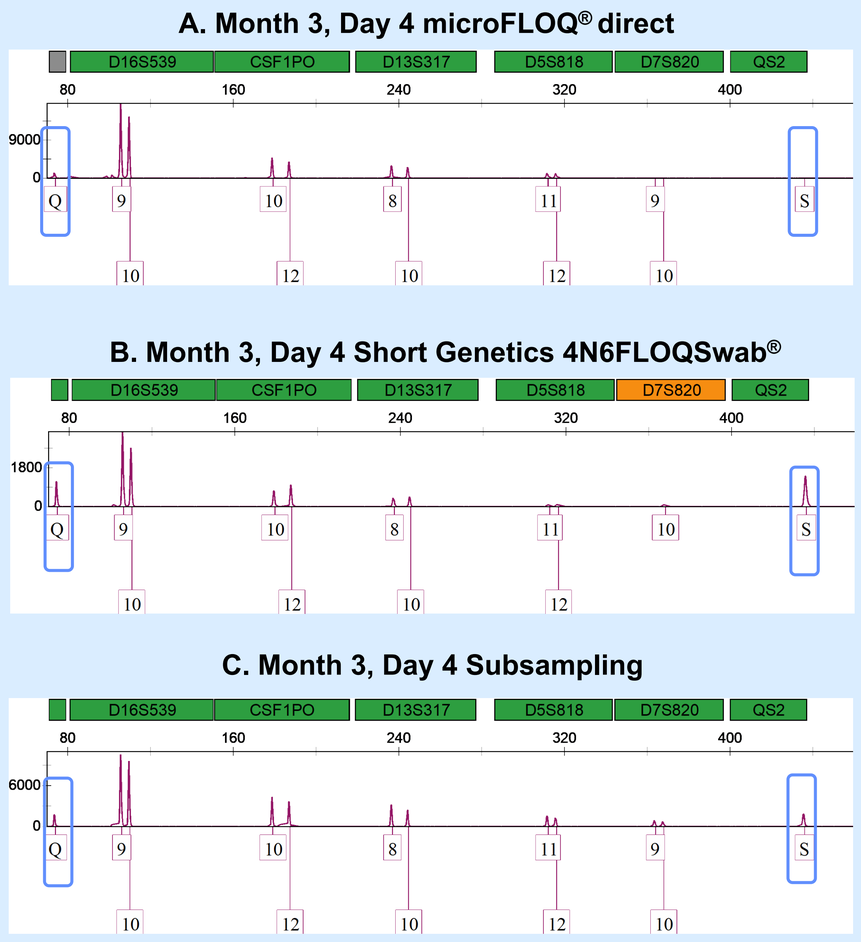

Figure 7: Electropherogram results comparing the purple dye channels for day 4 of the microFLOQ® swab (A), the Short Genetics 4N6FLOQSwab® (B), and the subsampling swab (C).

CONCLUSIONS

- Traditional and direct PCR methods were comparable up to day 10 depending on the sample (full profiles obtained with both methods for thigh samples) for up to 3 months of storage at RT.
- The hybrid strategy using the traditional Genetics 4N6FLOQSwabs® to store DNA and the microFLOQ® swabs to subsample and process the DNA allowed for rapid processing without total consumption of the sample.
- Processing of the microFLOQ® swabs was improved by the addition of a short pre-treatment step.
- Future research and testing is recommended with direct-to-casework buffers to overcome inhibition present in forensic and DVI-type samples processed via direct PCR.

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