Direct PCR using microFLOQ® Direct Swabs with a Modified QIAGEN Investigator 24plex GO! Protocol from Decomposing Human Remains for DVI Applications

Coral Loockerman, MS, Sheree R. Hughes, PhD, Rachel Houston, PhD

Department of Forensic Science
Sam Houston State University
Huntsville, TX, USA

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FSF Emerging Forensic Scientist Award
Paper Presentation

Disclosure

There is no real or apparent conflicts of interest related to the content of this presentation.

Disclaimer

- Some images may be graphic or disturbing
- Photographs of human remains are **NOT** permitted

Background

Challenges with DVI

- Remote locations
- Temperature
- Lack of refrigeration
- Cost
- Transportation of human remains
- Decomposition and age of remains
- Fragmentation

An attempt to slow putrefaction of tsunami victims at Ban Muang Temple, Thailand (Jan. 2, 2015)

DNA Collection Methods

- Bone:
  - Skeletonized remains
  - Resistant to degradation
  - Challenging processing
  - Lengthy demineralization
- Tissue:
  - Ease of collection
  - Challenging storage (decomposition)
  - Long lysis

DNA Collection Methods

- Swabs: an *alternative* collection method when tissue is available

- Swabbing red muscle:
  - Ease of collection
  - Shorter lysis than tissue
  - Re-cap for easy storage
  - Desiccation- storage at room temperature?

DNA processing methods

- Direct Amplification

~~Extraction~~

~~Quantification~~

Amplification

Capillary Electrophoresis

OSAC Research Needs Assessment Form

Title of research need: Assessment of specific classes of evidence types to determine the necessity to quantify DNA before amplification of human autosomal STR loci

Keywords: DNA quantitation, trace DNA, direct PCR,

Submitting subcommittee(s): BDRIC **Date Approved:** 8/25/16

(If SAC review identifies additional subcommittees, add them to the box above.)

Background information:

1. Description of research need:

The current quality assurance standard (QAS) for forensic testing laboratories requires that human DNA quantitation is attempted for all forensic unknowns. This requirement poses a problem for evidence types expected to yield low amounts of DNA such as DNA swabs from cartridge casings, other touched objects, or single fingermarks. It has been shown that direct PCR amplification without prior DNA extraction can improve the DNA typing success rate, for example for touched fabric and fired cartridge cases. In the example of fired cartridge cases, rarely, if ever, will greater than 1ng of DNA be recovered. However, due to the QAS requirements, an extraction is performed solely to be able to perform the quantitation step prior. At this point, the entire extract typically will be concentrated and the entire volume used during the amplification. Unfortunately, it has been demonstrated numerous times that a significant portion of DNA is lost during DNA extraction and concentration. If there is no value to performing the extraction and quantitation, it would seem

Challenges of Direct Amplification

- Lacks purification step (inhibitors)
- Lacks quantitation step
  - FBI Quality Assurance Standards

STANDARD 9.4 The laboratory shall quantify the amount of human DNA in forensic samples prior to nuclear DNA amplification. Quantitation of human DNA is not required for casework reference samples if the laboratory has a validated system that has been demonstrated to reproducibly and reliably yield successful DNA amplification and typing without prior quantitation.

Quality Assurance Standards for Forensic DNA Testing Laboratories
Effective September 1, 2011

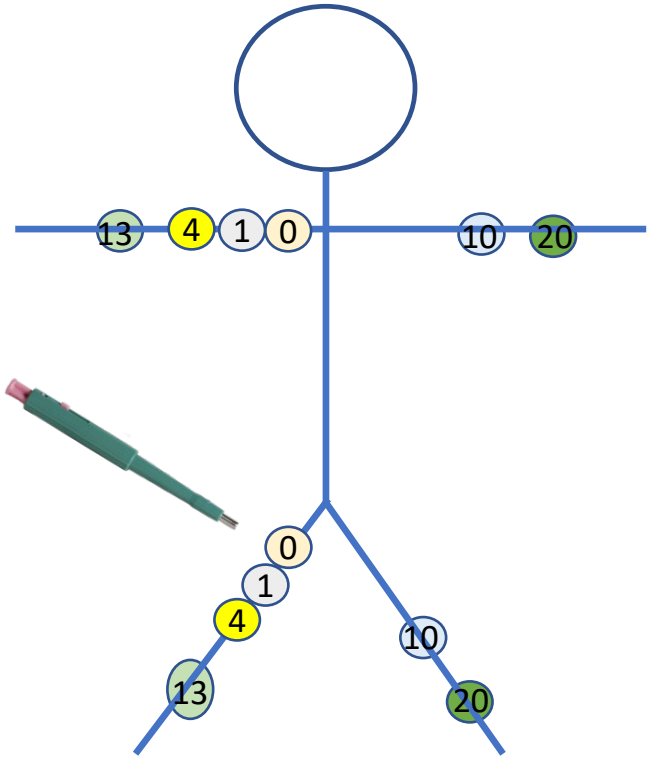
Research Questions:

1. Can swabs be used to collect and store DNA at room temperature over time?
2. Can microFLOQ® swabs be used to collect and directly amplify DNA from a decomposing cadaver?

Materials and Methods

Collection

- Collect duplicate samples from arm and leg
- Collection on days 0, 1, 4, 10, 13, 20
- Swabs collected in triplicate for **3 storage points**
- **3 swab types:** short genetic, long genetic, and microFLOQ® direct
- Total of 54 swabs per day
- **Tissue control** collected



Swab Types

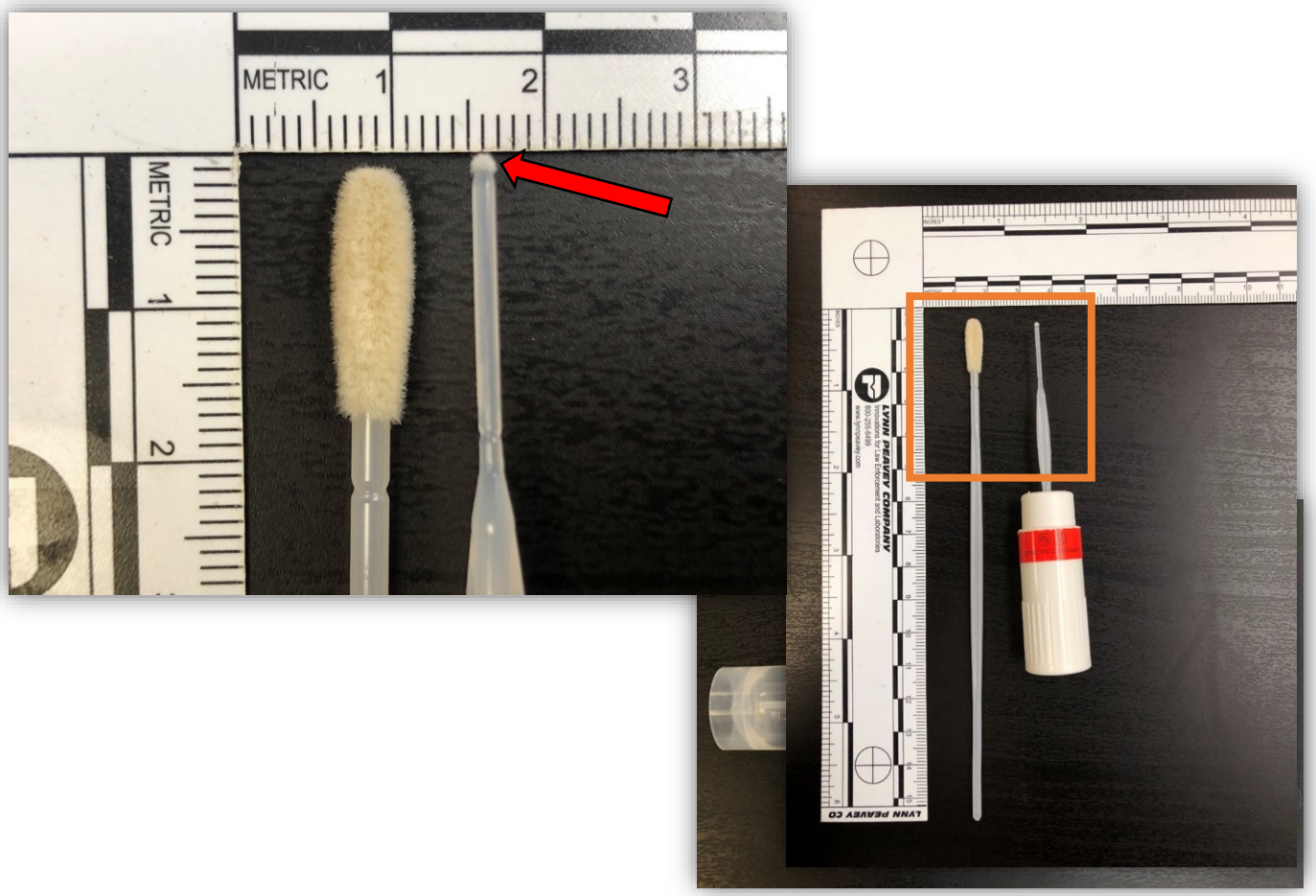
4N6FLOQSwab® Genetics Short	4N6FLOQSwab® Genetics Long	microFLOQ® Direct
		
Desiccant	None	Desiccant & lysis

*images courtesy of www.copangroup.com

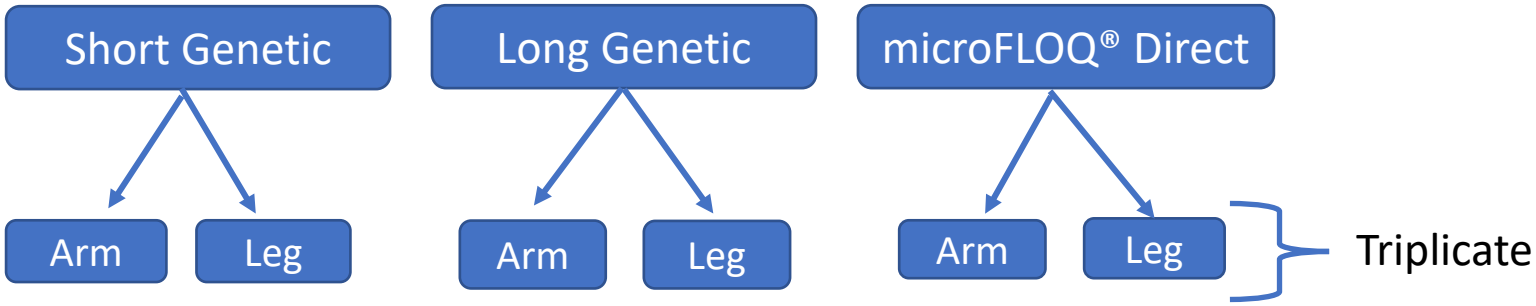
microFLOQ® Direct swabs

- Specifically designed for direct amplification
- Miniature nylon-flock swab
- Breakable swab head
  - Lysing agent on swab head

microFLOQ® Direct swabs



Sample Types



Day 10 arm

Day 10 leg

Storage

- Swabs capped and stored in darkness at room temperature
- Swabs processed in three groups: overnight drying, storage for 1 month, and storage for 3 months

Processing

- microFLOQ®
  - ✓ Direct amplification using Investigator 24Plex GO! Kit (QIAGEN)

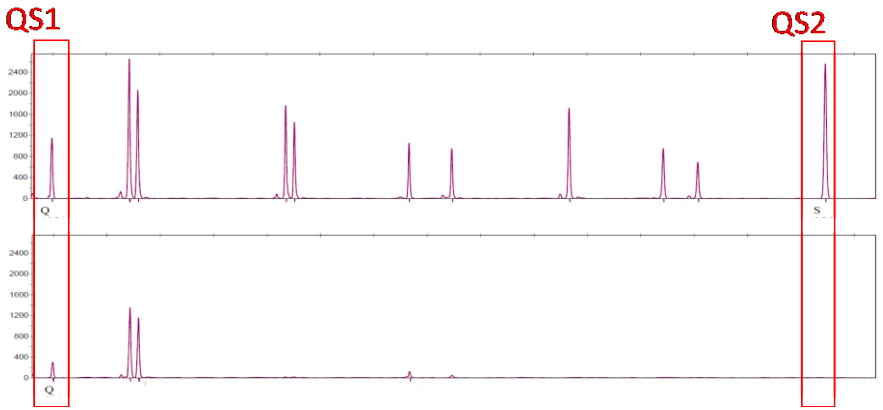
Total processing time: 2 hours 15 minutes

- Traditional
  - ✓ Extraction using EZ1 DNA Investigator Kit (QIAGEN)
  - ✓ Quantification using Investigator Quantiplex Pro Kit (QIAGEN)
  - ✓ Amplification using Investigator 24Plex QS Kit

Total processing time: 6 hours 48 minutes

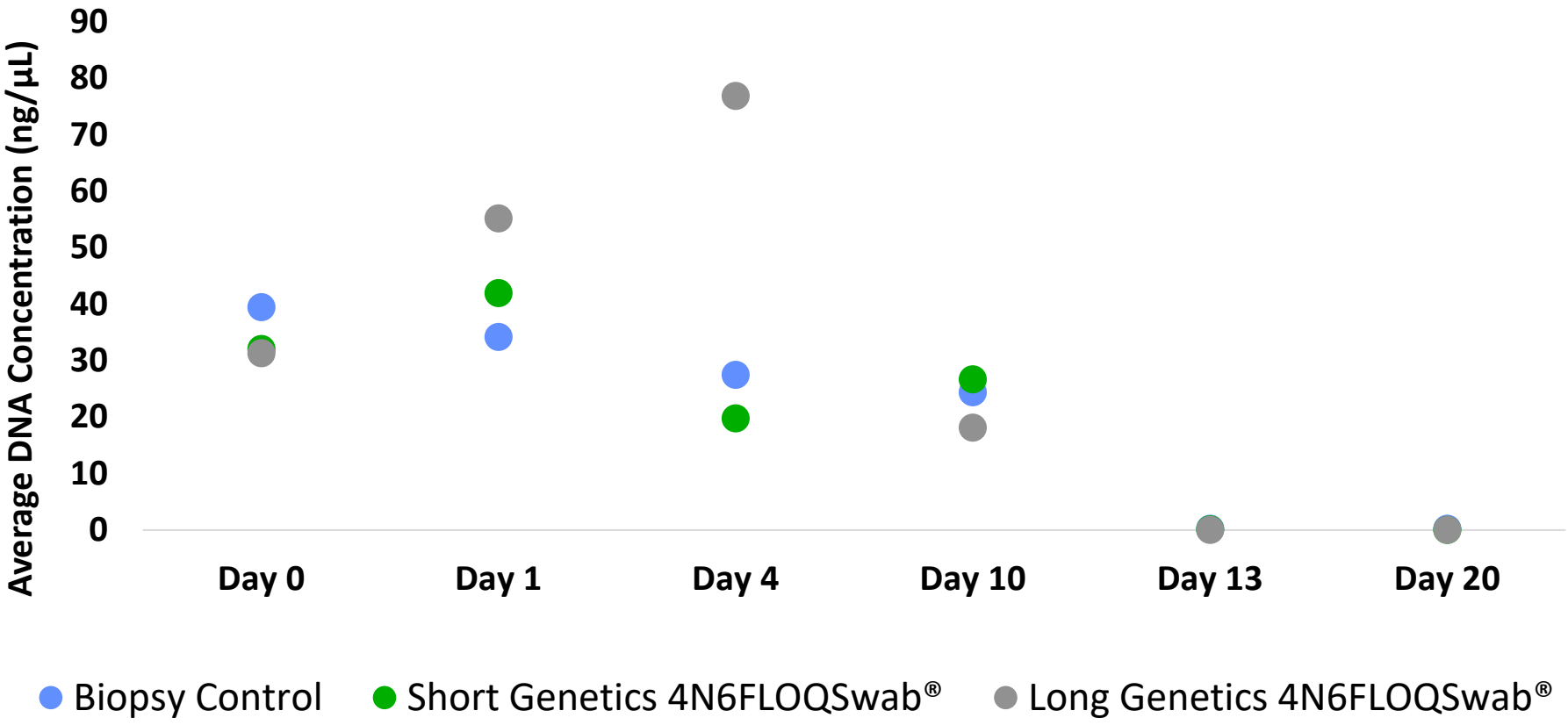
Quality Sensors

- Synthetic DNA
  - QS1 = 74 bp; QS2 = 435 bp
- Substantial levels of PCR Inhibition when S/Q ratio <20%
- May be used as a threshold to guide *rework strategy*



Results

Biopsy & swab DNA concentrations



- DNA concentration dropped severely after 13 days of decomposition

STR completeness

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

*N=6 replicates per day/time point, data is averaged

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

- Full STR profiles were still obtained with biopsy controls and Genetics 4N6FLOQSwabs®
- No significant difference was observed in profile completeness across storage times

Challenges

Inhibition in direct PCR samples

Artifacts:

microFLOQ® DNA input not normalized

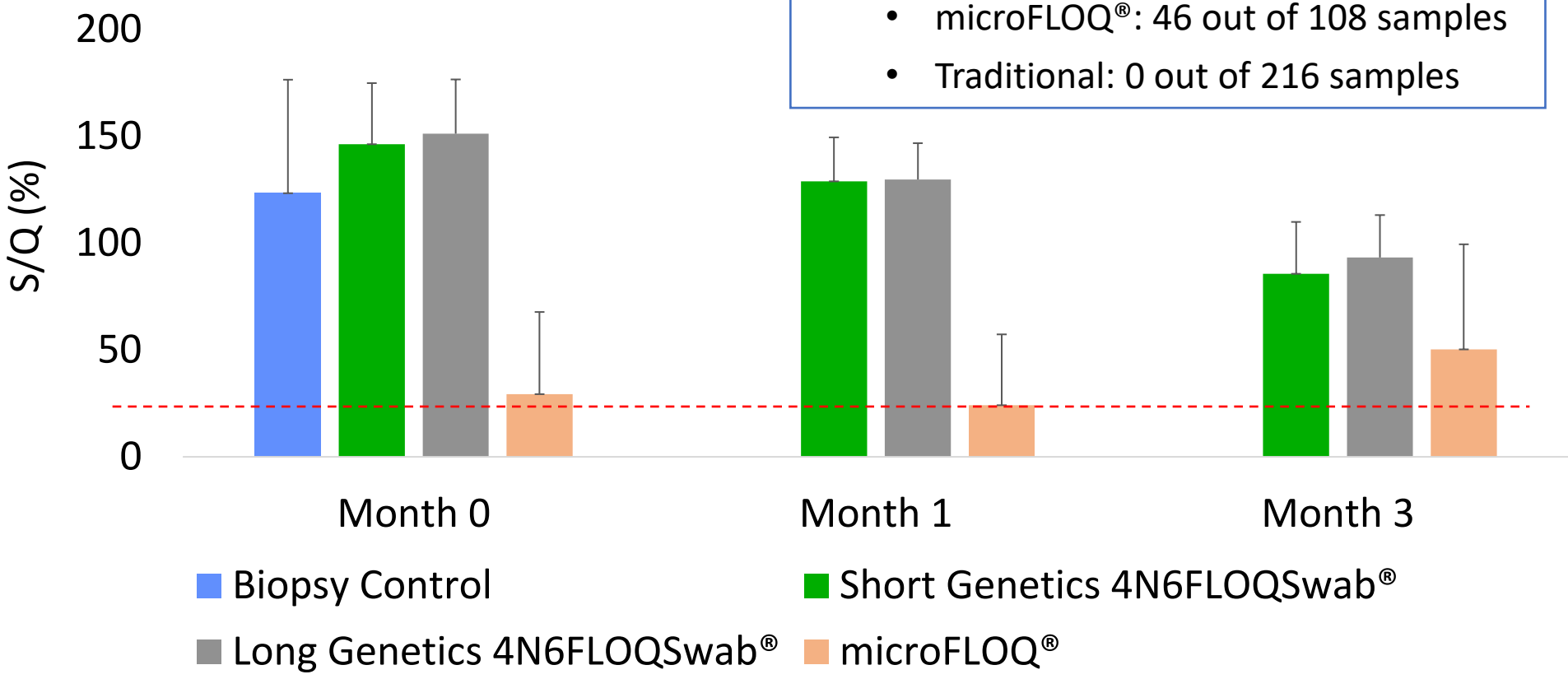
Optimization

Temperature (°C)	Time	Cycle number
98	30 sec	
64	40 sec	3
72	5 sec	
96	10 sec	
61	40 sec	23
72	5 sec	
68	4 min	
60	4 min	1
10	∞	

- Wash step added + lysis with GO! Lysis buffer
- Modified cycling parameters from recommended (longer adenylation step)
- Rapid processing (1 hour of drying) can reduce inhibition

Inhibition

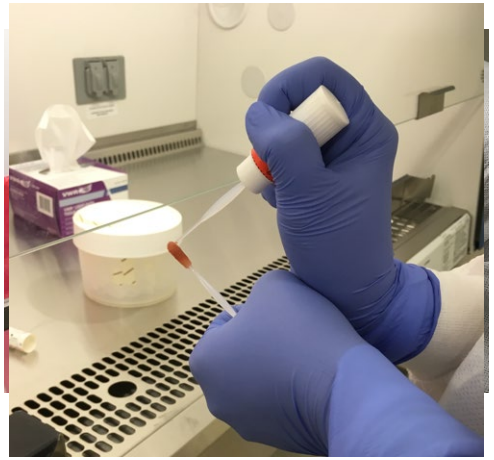
- **S marker dropout**:
  - microFLOQ®: 46 out of 108 samples
  - Traditional: 0 out of 216 samples



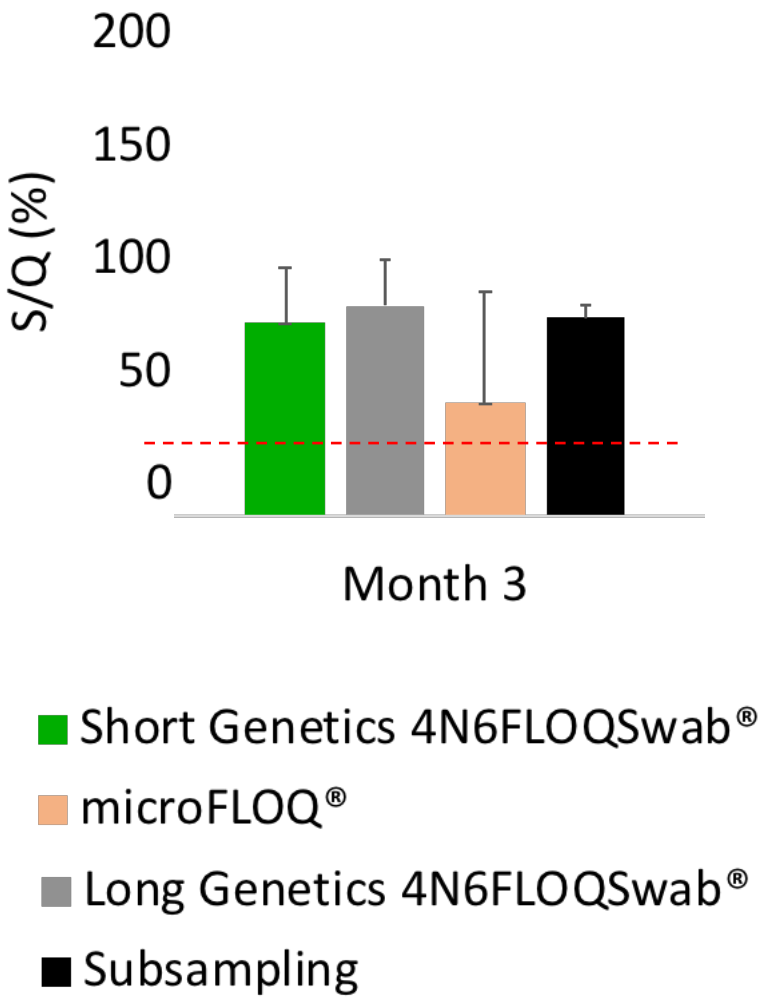
*Average QS scores across 3 months for all swab types and biopsy tissue. Biopsy, N=12; Short Genetics 4N6FLOQSwab®, microFLOQ® direct, Subsampling, N=36. Error bars represent standard deviation

Subsampling

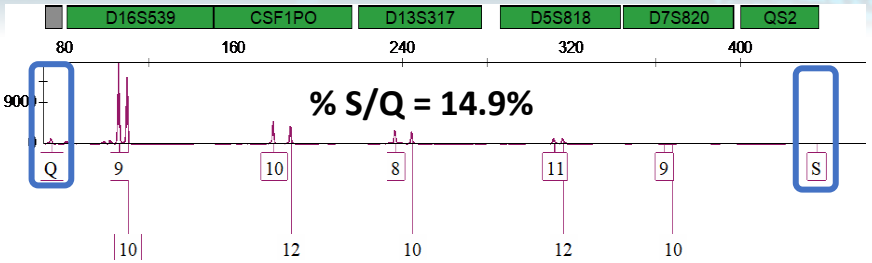
- Subsampling is an alternative collection/processing combination for samples
  - Traditional swabs collected and swabbed as normal, later microFLOQ® used to subsample swab
  - Original swab not consumed
  - Fast microFLOQ® processing, long-term storage, re-testing of samples



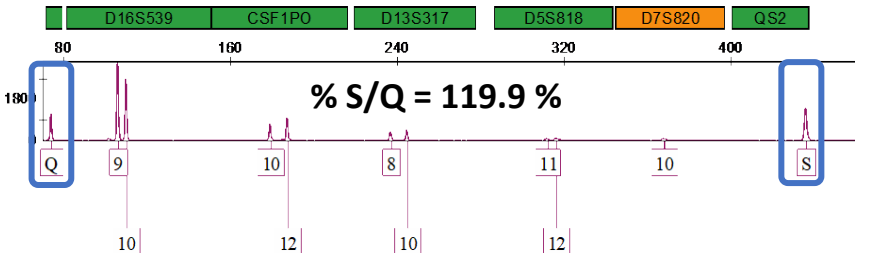
Subsampling results



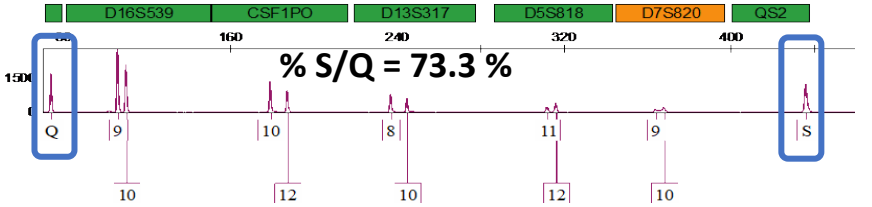
A. Month 3, Day 4 microFLOQ® direct



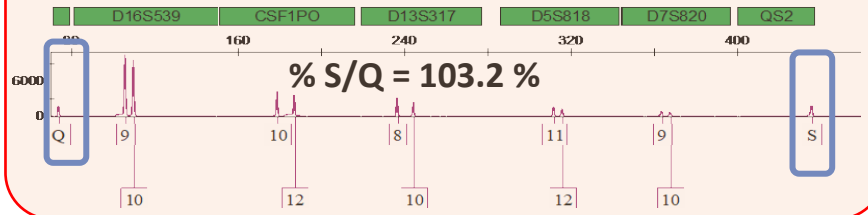
B. Month 3, Day 4 Short Genetics 4N6FLOQSwab®



C. Month 3, Day 4 Long Genetics 4N6FLOQSwab®



D. Month 3, Day 4 Subsampling



Subsampling STR completeness

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

*N=6 replicates per day/time point, data is averaged

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

- A reduction in PCR inhibition observed with the subsampling method compared to direct swabbing with the microFLOQ® swabs
- Improved profile completeness with subsampling method

Additional results

- Tested the relative tolerance of other STR chemistries with this inhibition
  - Similar inhibition observed with NGM Detect™ (ThermoFisher) and PowerPlex® Fusion 6C (Promega)

- Determined that *Quasi-Direct* method necessary for inhibited cadaver samples

Investigator® Casework GO! Buffer

- Utilized buffer for quick (30 min.) extraction in small volume (20 µL) with microFLOQ™ direct swabs
  - Facilitated quantification and clean up of cadaver swab samples

Day	Biopsy Control	microFLOQ® direct			Subsampling			Casework GO! Buffer
		Month 0	Month 1	Month 3	Month 0	Month 1	Month 3	
0								
1								
4								
7								
10								
11								
13								
14								
15								
20								

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

Conclusions

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.
- ✓Direct-to-casework buffer able to overcome inhibition present in DVI-type samples processed via direct PCR.

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Questions

Rachel Houston

rmh034@shsu.edu