Substance Abuse and Mental Health Services Administration

SAMHSA

www.samhsa.gov • 1-877-SAMHSA-7 (1-877-726-4727)

DEPARTMENT OF HEALTH & HUMAN SERVICES • USA

Behavioral Health is Essential To Health

Prevention Works

Treatment is Effective

People Recover

Substance Abuse and Mental Health Services Administration
SAMHSA
www.samhsa.gov • 1-877-SAMHSA-7 (1-877-726-4727)

DNA in Urine and Oral Fluid

Robert M. White, Sr., Ph.D., D.A.B.C.C. , F.-A.B.F.T.
Rockville, Maryland

September 19, 2017

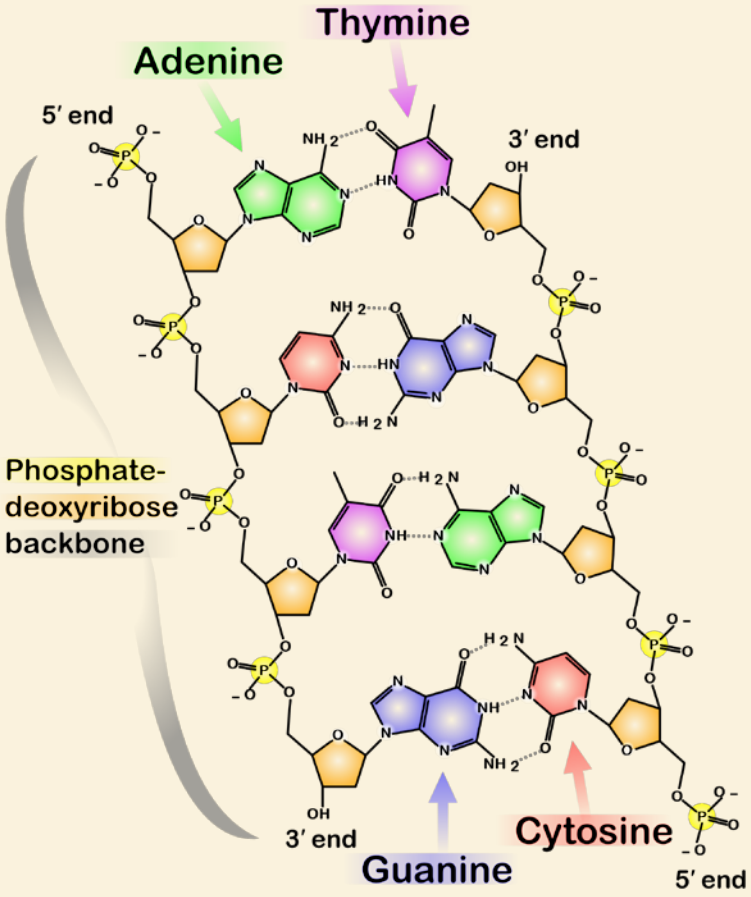
DEPARTMENT OF HEALTH & HUMAN SERVICES · USA

DNA – A Very Brief Review

- DNA = Deoxyribonucleic Acid.
- Adenine (A), Thymine (T), Cytosine (C), and Guanine (G) linked through phosphate bonds.
- Exists in humans as nuclear (cell nucleus) and mitochondrial (mtDNA; cytosol).
- DNA is the blueprint for proteins.
- Human nuclear DNA is double-stranded.
- About 99.7-99.9% identical from individual to individual.

Substance Abuse and Mental Health Services Administration SAMHSA

STRUCTURE OF DNA



Source: Wikipedia "DNA" last accessed 8/6/2017.

Basic Dogma

DNA

↓

RNA

↓

PROTEIN

Substance Abuse and Mental Health Services Administration
SAMHSA

Individual Differences

- SNPs (Single Nucleotide Polymorphisms, “snips”).
- HLA-DQA1 + PM.
- STRs (Short Tandem Repeats).

Substance Abuse and Mental Health Services Administration
SAMHSA

Most Common STRs + Amelogenin

Marker	Repeat	Marker	Repeat
D3S1358	[TCTG][TCTA]	D16S539	GATA
TH01	TCAT	CSF1PO	TAGA
D21S11	[TCTA][TCTG]	Penta-D	AAAGA
D18S51	AGAA	vWA	[TCTG][TCTA]
Penta-E	AAAGA	D8S1179	[TCTA][TCTG]
D5S818	AGAT	TPOX	GAAT
D13S317	TATC	FGA	CTTT
D7S820	GATA	Amelogenin	N/A - ♀/♂

Substance Abuse and Mental Health Services Administration SAMHSA

Proposed Study

- Determine the approximate amount of degradation DNA might experience under routine storage conditions typical of a drug testing laboratory.
  - RTI IRB-approved.
  - 5 Donors (2 Females; 3 Males).
  - All samples self-collected.
  - Collected “A” and “B” urine & oral fluid.
  - NO DRUG TESTING WAS INCLUDED IN THE STUDY.

Substance Abuse and Mental Health Services Administration SAMHSA

Proposed Study (cont'd)

- Samples were stored on the RTI campus in an air-conditioned office prior to transportation to testing lab.
- Samples were transported to the testing laboratory (LabCorp) in Burlington by a courier car.
- FedEx, UPS and commercial air were not part of the transportation system.

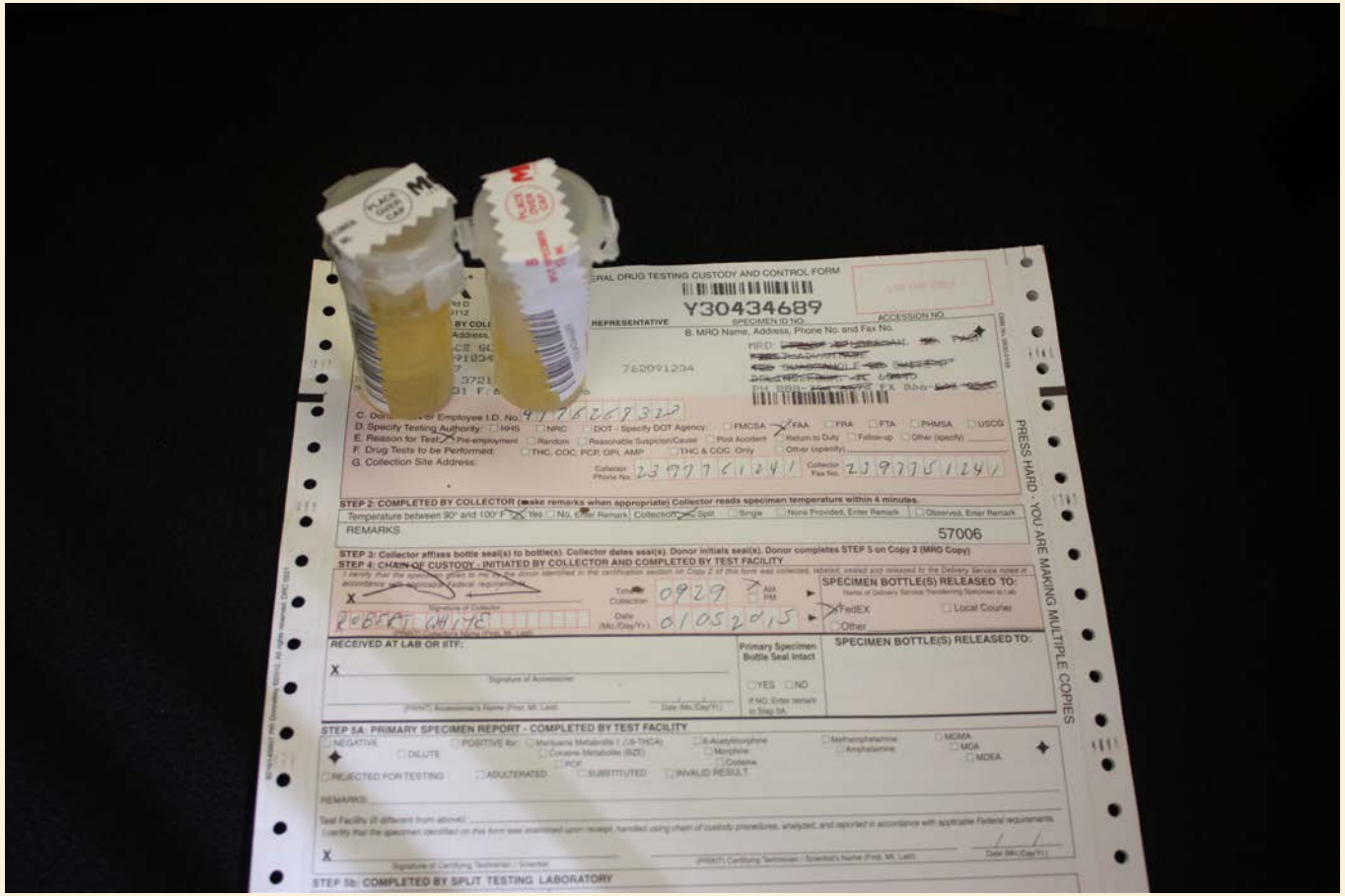
Substance Abuse and Mental Health Services Administration SAMHSA

Urine

- Contains some nuclear DNA mostly due to the presence of intact epithelial cells.
- Hypertonic.
- Not a favorable environment for DNA or intact cells.
- Analyzed for STRs at 5 days post collection (“A” Bottles) and 35 days post-collection (“B” Bottles; frozen upon accessioning).

Substance Abuse and Mental Health Services Administration SAMHSA

Urine Collection

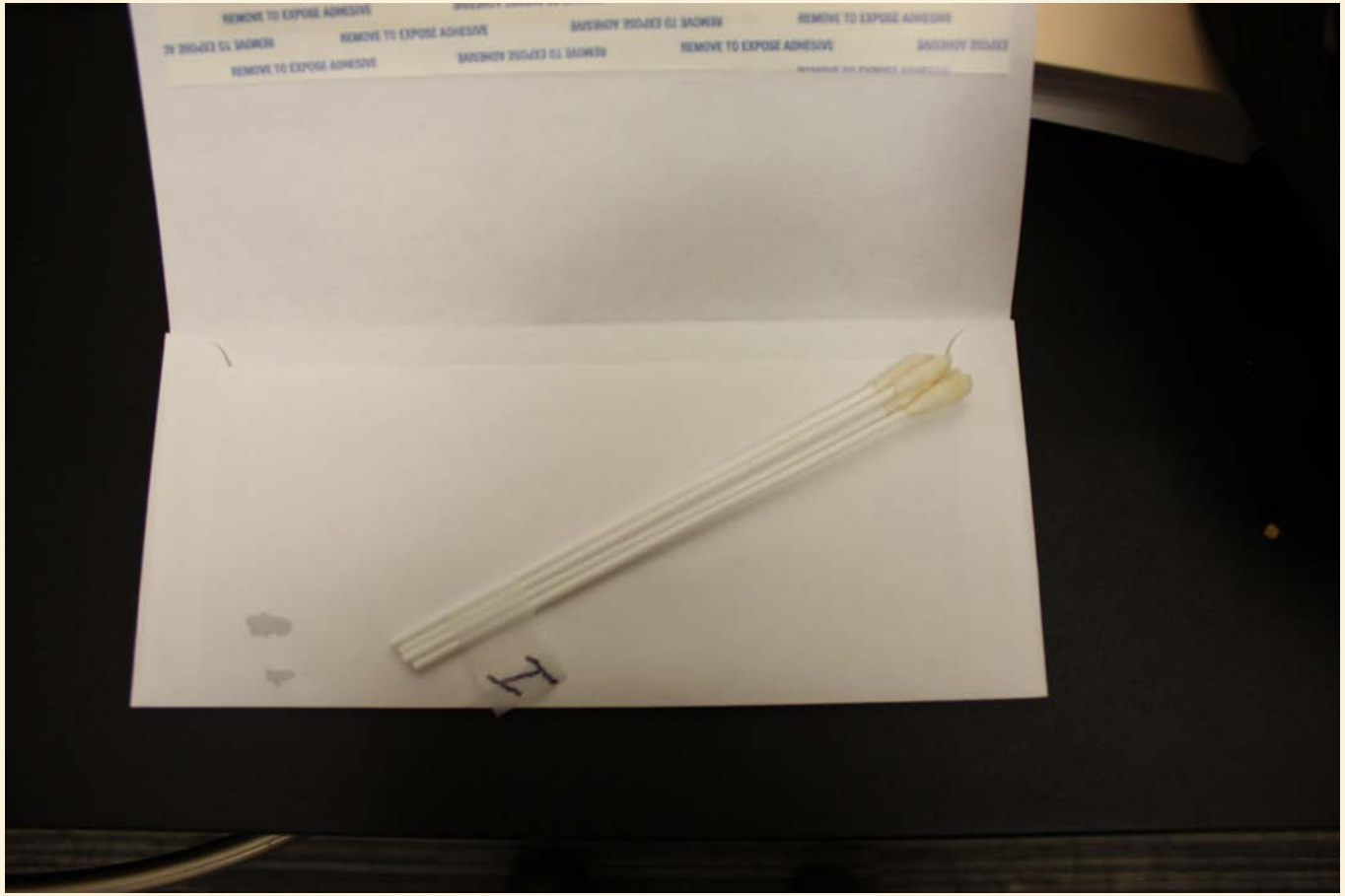


Oral Fluid

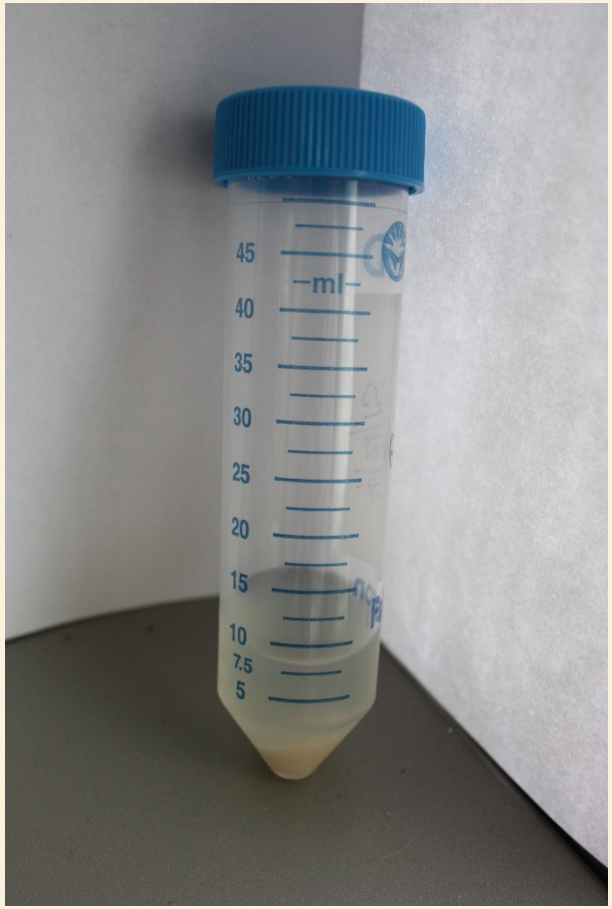
- Originates from the same cavity from which buccal swabs for DNA are obtained.
- Neat Oral Fluid and buccal swabs were analyzed 5 days post-collection and 35 days post-collection.
- Pad-type devices were analyzed 5 days post-collection and 39 days post-collection.

Substance Abuse and Mental Health Services Administration
SAMHSA

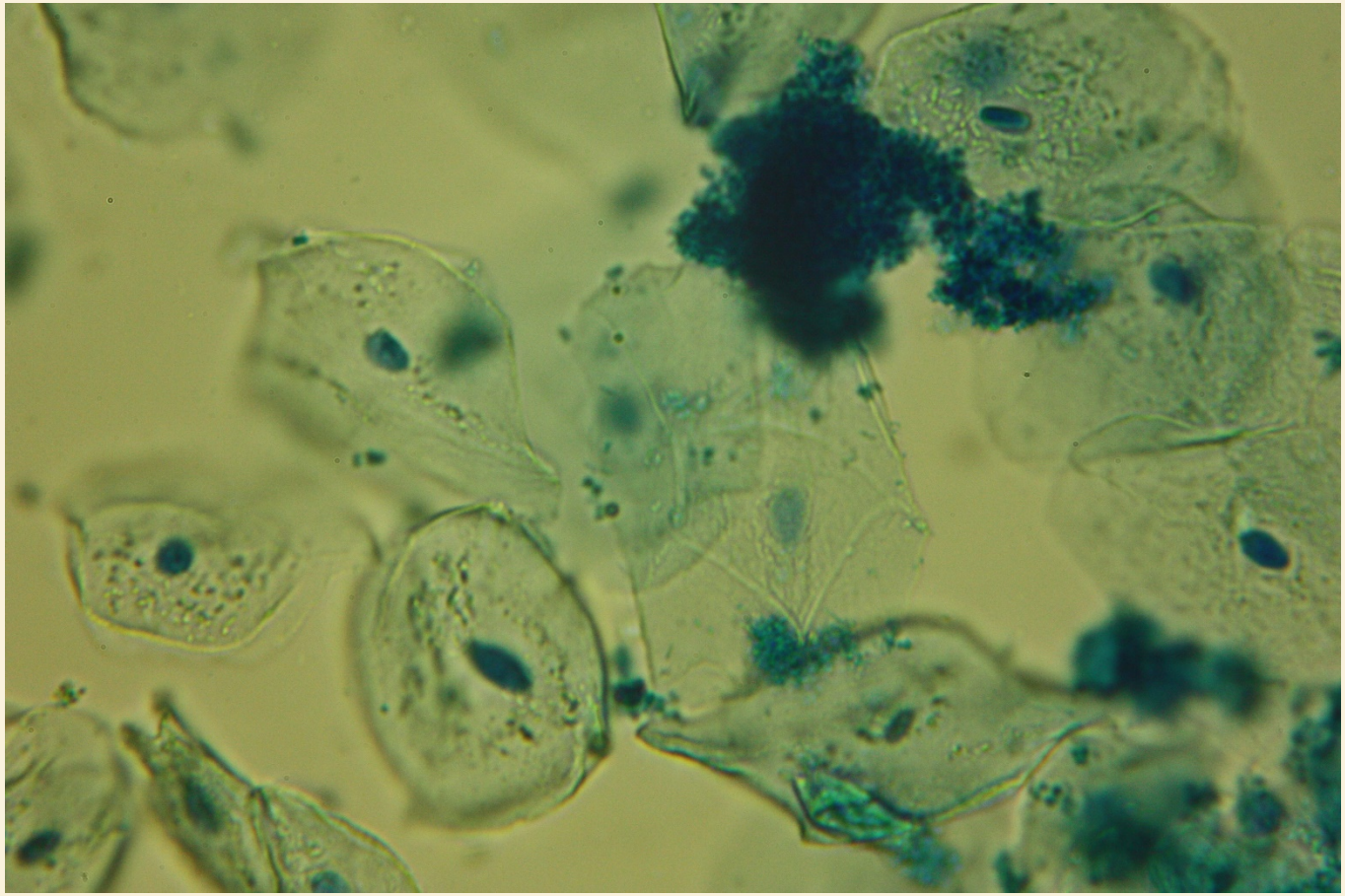
Buccal Swabs



Neat Oral Fluid



Neat Oral Fluid



Neat Oral Fluid Collector

Substance Abuse and Mental Health Services Administration
SAMHSA

“Pad type” OF Collectors

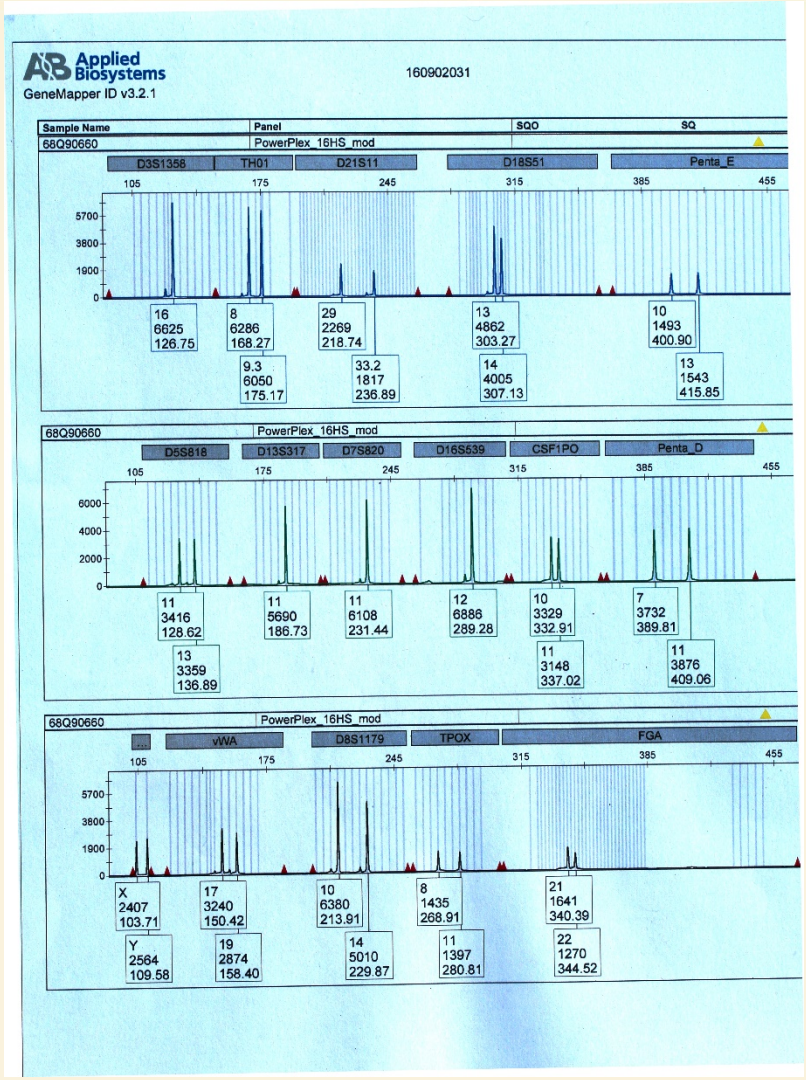
- Three commercially available collectors.
- Designated “A,” “B,” and “C.”

Experimental Methods

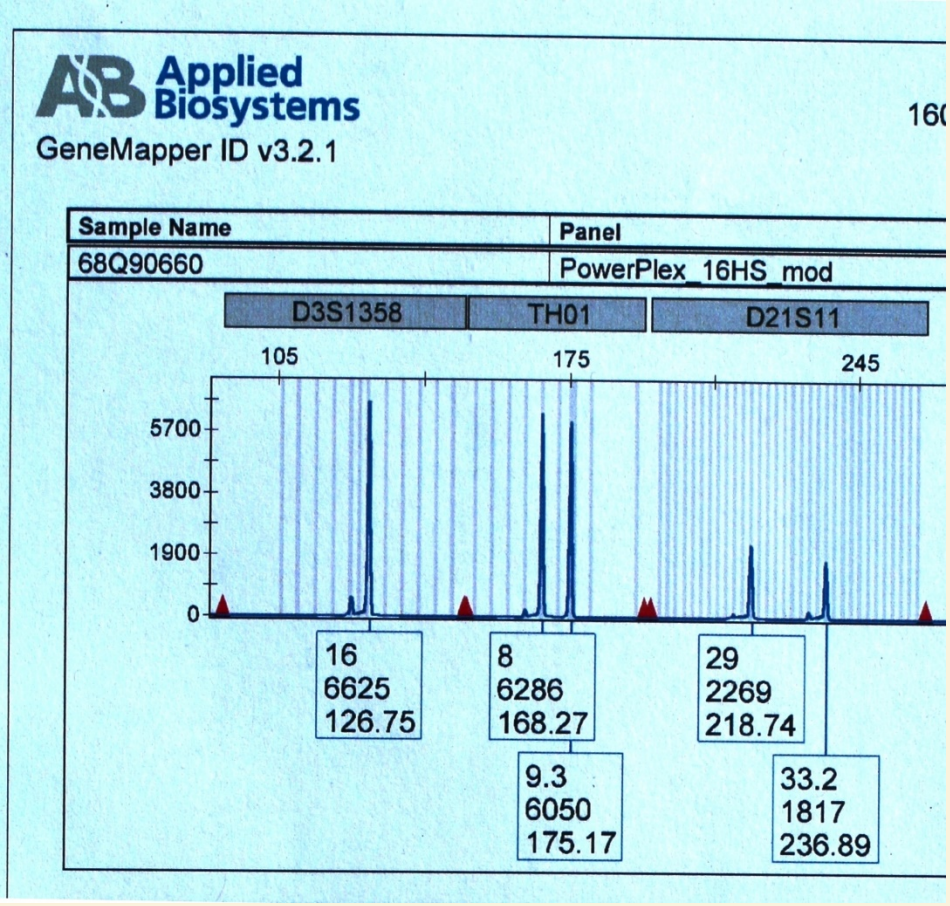
- DNA extraction - QIAmp® DNA Investigator.
- DNA Quantitation – QuantiFluor® One dsDNA System.
- Capillary electrophoresis – 3130xl Genetic Analyzer.
- 15 STRs + amelogenin - PowerPlex® 16HS.

Substance Abuse and Mental Health Services Administration SAMHSA

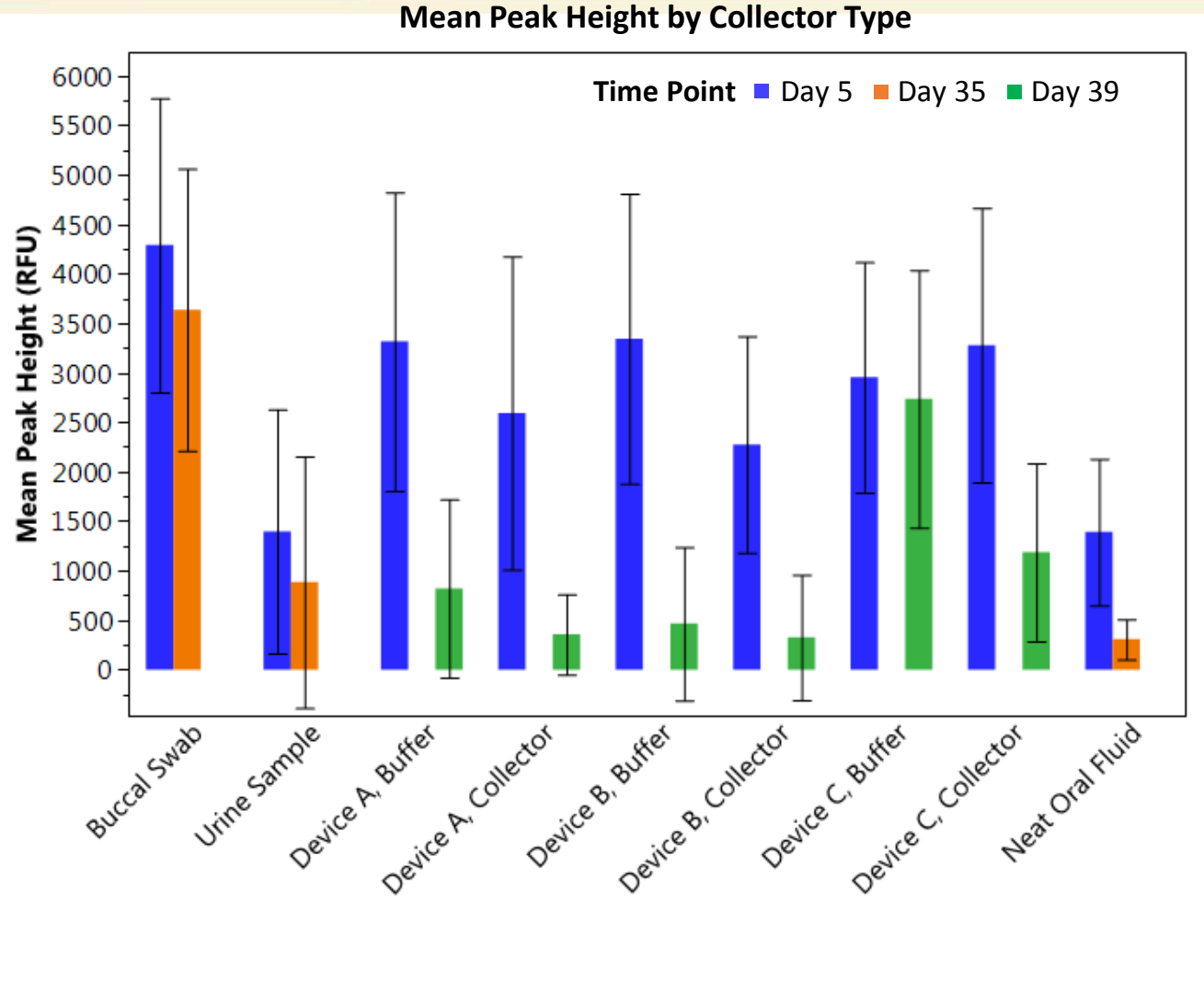
Electropherogram



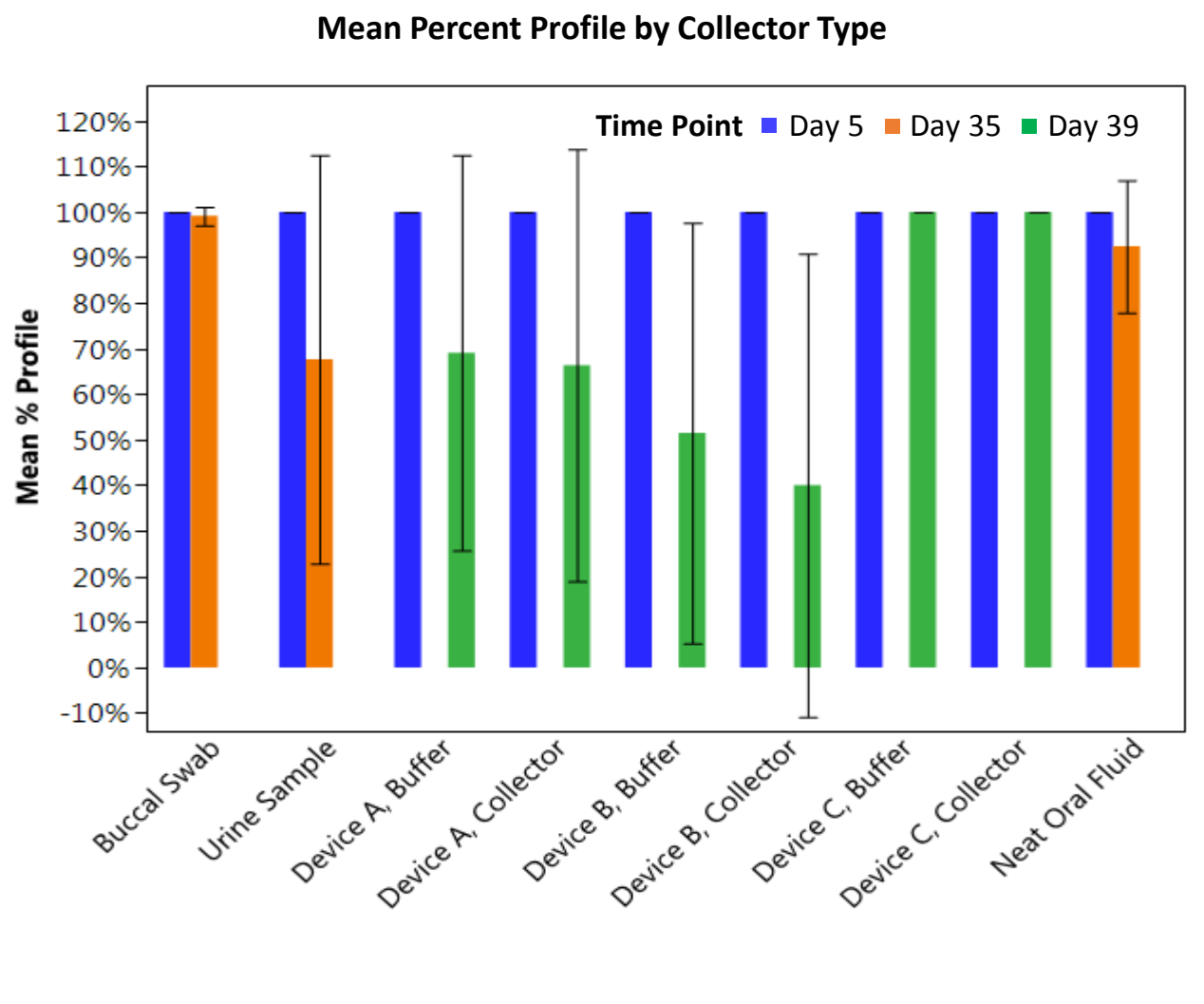
Electropherogram (cont'd)



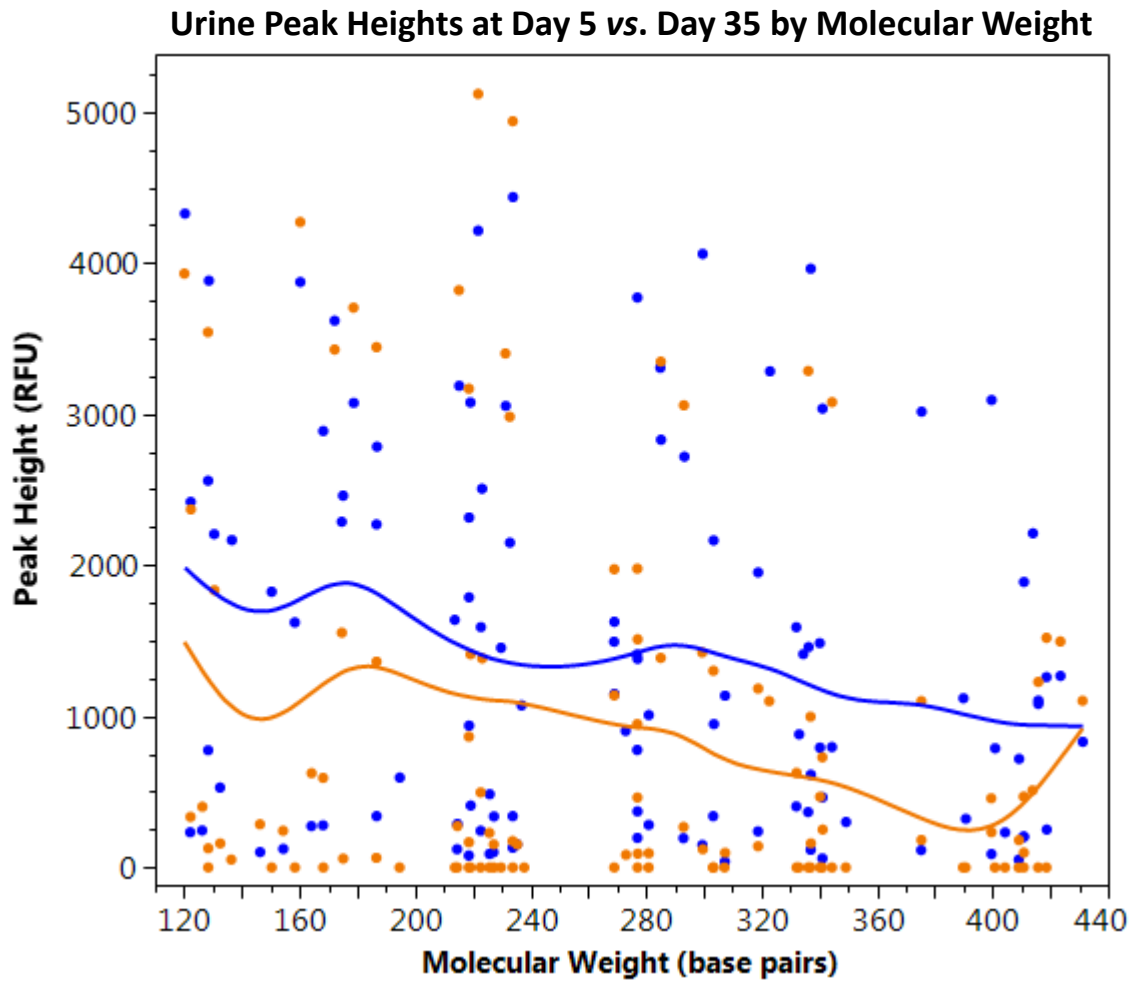
Results


Mean Peak Height by Collector Type
Time Point
Day 5
Day 35
Day 39
Mean Peak Height (RFU)
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Buccal Swab
Urine Sample
Device A, Buffer
Device A, Collector
Device B, Buffer
Device B, Collector
Device C, Buffer
Device C, Collector
Neat Oral Fluid

Results (Cont'd)



Urine Results



Comparison P-values

Matrix	Comparison	P-Value
Buccal Swab	Day 5 *vs.* Day 35	0.0009
Urine	Day 5 *vs.* Day 35	0.0023
Neat Oral Fluid	Day 5 *vs.* Day 35	0.0023
"A"	Buffer, Day 5 *vs.* Day 39	<0.0001
	Pad, Day 5 *vs.* Day 39	<0.0001
	Buffer *vs.* Pad, Day 5	0.0013
	Buffer *vs.* Pad, Day 39	0.0008
"B"	Buffer, Day 5 *vs.* Day 39	<0.0001
	Pad, Day 5 *vs.* Day 39	<0.0001
	Buffer *vs.* Pad, Day 5	<0.0001
	Buffer *vs.* Pad, Day 39	0.8345
"C"	Buffer, Day 5 *vs.* Day 39	0.1870
	Pad, Day 5 *vs.* Day 39	0.0004
	Buffer *vs.* Pad, Day 5	0.6336
	Buffer *vs.* Pad, Day 39	<0.0001

Substance Abuse and Mental Health Services Administration SAMHSA

Conclusions

- Urine – “A” Bottles were acceptable 5-6 calendar days post-collection; however, a complete profile was not obtained in 40% of cases after freezing “B” Bottles until day 35 post-collection.
- Neat Oral Fluid – Similar to urine but with a complete profile at 35 days post-collection in only 4/5 cases.

Substance Abuse and Mental Health Services Administration SAMHSA

Conclusions (“Pad-type” Devices)

- The oral fluid pad eluent and the buffer-preservative produced a complete profile for all collectors for all donors 5 days post-collection.
- However, at 39 days post-collection, only the “C” pad and buffer-preservative produced a complete profile after 35 days storage.
- The pilot study provides no stability data beyond 35 days for frozen urine and oral fluid.

Substance Abuse and Mental Health Services Administration SAMHSA

Conclusions (cont'd)

- The pilot study provides no stability data beyond 39 days for pad-type collectors.
- Whether usable DNA can be extracted from a pad or its buffer/preservative after extended storage needs to be the subject of a study that employs a longer storage time.

Substance Abuse and Mental Health Services Administration SAMHSA

Project Team

Bob White (RTI)
John Mitchell (RTI)
Dale Hart (RTI)
Amy Evans (RTI)
Meredith Meadors (RTI)
Sara Norsworthy (RTI)
Eugene Hayes (SAMHSA)
Ron Flegel (SAMHSA)
George Maha (LCA)
Megan S. MacKenzie (LCA)
Erin C. Hall (LCA)
Kelley Rogers (LCA)

Substance Abuse and Mental Health Services Administration SAMHSA

Thank You

Questions? Comments?

Substance Abuse and Mental Health Services Administration
SAMHSA