

Sample Stabilization and Purification Devices for Superior Forensic DNA Storage, Purification and Profiling

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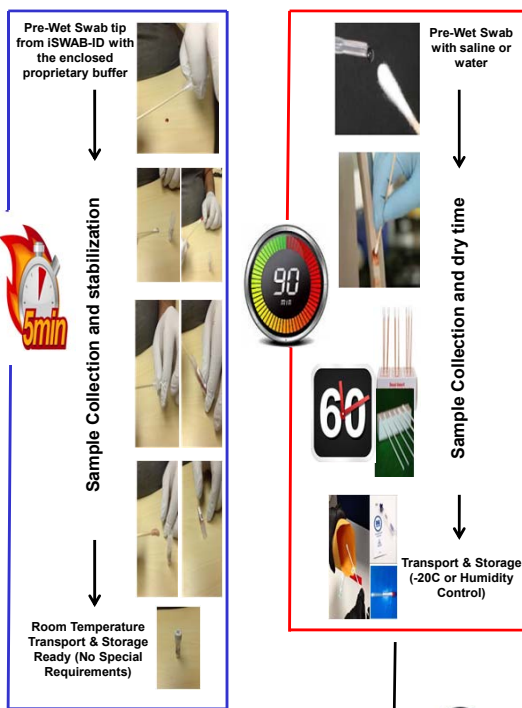


1. Introduction

Maintaining the integrity of evidentiary and reference samples until DNA analysis is performed continues to be a major challenge - especially when samples stay in transit during transportation and/or need to be stored for extended periods, which can be hours to months if not years.

Mawi has developed an efficient sample collection system, iSWAB-ID, which enables long term room temperature stabilization of the collected sample at the point of collection, while ensuring proper chain of custody. This system allows for maximizing sample recovery and obtaining human DNA compatible with ID profiling assays. DNA extraction can be performed using any commercially available whole blood extraction chemistry such as AnaPrep systems from BioChain.

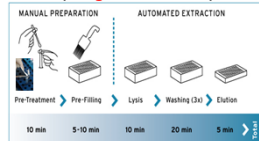
iSWAB-ID Collection vs. Traditional Swab Collection



Process (2-3 Runs/iSWAB-ID Sample)



Process (Single Run/Swab)



2. Objectives

- To assess the efficiency of iSWAB-ID in the collection and stabilization of both reference and evidentiary samples by assessing the usability of purified DNA in human ID profiling assays.
- To assess the efficiency of AnaPrep automated nucleic acid extractor in purifying human DNA from reference and evidentiary samples in a forensic setting

3. Materials & Methods

- Reference and mocked samples were collected with iSWAB-ID according to manufacturer's instructions.
- All collected samples were transported at room temperature
- DNA was extracted from 100µL aliquots of iSWAB-ID stored at room temperature for two days or two weeks using AnaPrep 12 Blood DNA extraction kit (PN # z1322001)
- Swabs where processed with QiaAMP min Blood kit (PN # 51104, Buccal Swabs extraction protocol) to purify any left over DNA post iSWAB-ID collection
- Extracted DNA was analyzed by Nanodrop spectrophotometer and the QIAGEN Investigator Quantiplex Kit (PN # 387016) to further confirm presence of amplifiable human DNA using ABI7500 Fast Real-Time PCR System.
- STR profiling and analysis was performed by Sorenson Forensics utilizing Promega PowerPlex 16 HS

4. Results

iSWAB-ID Recovers and Stabilizes Human DNA Efficiently from both Reference & Evidentiary Samples

DNA concentration from swabs post iSWAB-ID collection: ND

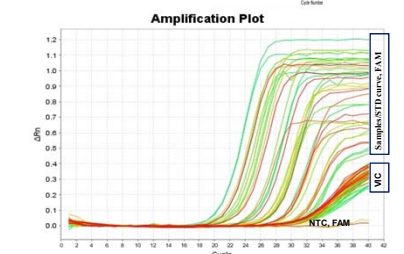
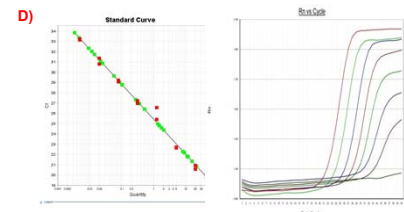
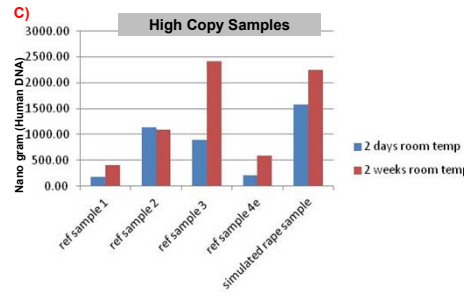
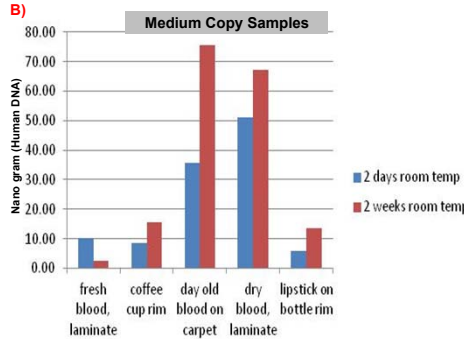
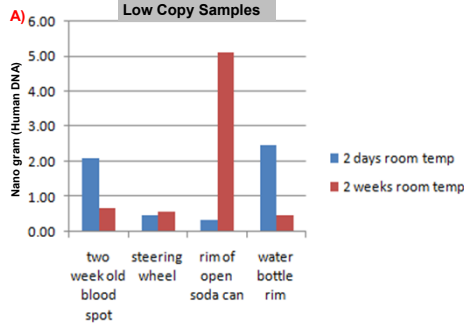


Fig 1. A selection of real-world, reference, and simulated evidence samples were collected in iSWAB-ID devices and stored at room temperature. DNA was extracted from 100 µL aliquots, using the AnaPrep Blood DNA extraction kit on AnaPrep 12 instrument, either two days or two weeks after collection. In all cases, PCR-amplifiable Human DNA was recovered from stabilized samples after storage for two weeks at room temperature. All samples were quantified by QIAGEN Investigator Quantiplex Kit targeting Human DNA. A) Low copy samples, B) Medium copy samples, C) High copy samples, D) Amplification blot for all samples including standard curve, VIC, (two days & 2 weeks samples), ND: Not Detected

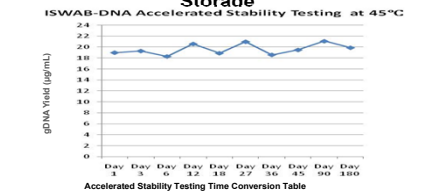
Based on Sorenson Forensics STR Profiling: Samples are Suitable for Comparison Purposes

Locus	Coffee Cup Rim		Reference Sample 1		Lip Stick	
	2 days	15 Days	2 days	15 Days	2 days	15 Days
AMEL	X,Y	X,Y	X	X	X	X
D3S1358	15,16	15,16	15,17	15,17	15,17	15,17
D1S1656	15,16	15,16	17,219,3	17,219,3	17,219,3	17,219,3
D2S441	15,16	15,16	14,17	14,17	14,17	14,17
D10S1248	15,16	15,16	14,17	14,17	14,17	14,17
D13S317	8,9	8,9	8,9	8,9	8,9	8,9
PENTA_E	12,17	12,17	5,12	5,12	5,12	5,12
D16S033	8,12	8,12	10,12	10,12	10,12	10,12
D18S11	14,23	14,23	13,18	13,18	13,18	13,18
D22S1048	16,20	16,20	17	17	17	17
CSF1PO	10,11	10,11	10,12	10,12	10,12	10,12
HTA_2	8,13	8,13	11,13	11,13	11,13	11,13
TH01	6	6	8,10	8,10	8,10	8,10
VWA	14,15	14,15	14,18	14,18	14,18	14,18
D5S11	29,32	29,32	30,32	30,32	30,32	30,32
D7S820	8,10	8,10	10	10	10	10
D5S818	12,13	12,13	12	12	12	12
TPOX	7,8	7,8	8,11	8,11	8,11	8,11
DY3S91	10	10	---	---	---	---
D12S301	12,13	12,13	12,13	12,13	12,13	12,13
D19S433	20,23	20,23	11,19	11,19	11,19	11,19
D15S102	13	13	15,2	15,2	15,2	15,2
FGA	23,26	23,26	22,23	22,23	22,23	22,23
D22S1048	15,16	15,16	16,18	16,18	16,18	16,18

Simulated Rape Sample

Locus	2 Days		15 Days	
	Item 9.0 Received Extract 14A - Q. LPCR/STR/Seq	Item 9.0 Received Extract 14A - Q. LPCR/STR/Seq	Item 10.0 Received Extract 14B - Q. LPCR/STR/Seq	Item 10.0 Received Extract 14B - Q. LPCR/STR/Seq
AMEL	X,Y	X	X,Y	X
D3S1358	17,18	17	17,18	17
D1S1656	15,16	15,16	15,16	15,16
D2S441	12,14	12,14	12,14	12,14
D10S1248	15,16,17	16,17	15,16,17	---
D13S317	8,11	8,11	8,11	8,11
PENTA_E	10,12,15,17	10,15	10,15	10,15
D16S033	8,12	8,12	8,12	8,12
D18S11	14,15,16	---	14,15,16	---
D19S433	18,22	18,22	18,20,22	18,22
CSF1PO	10,11,12	10,12	10,11,12	10,12
PENTA_D	10,14	10,14	10,14	10,14
TH01	6,9,9,3	---	6,9,9,3	---
VWA	14,15,16,18	16,18	14,15,16,18	---
D21S11	29	29	29	29
D7S820	9,12	9,12	9,12	9,12
D5S818	10,12,13	10,12	10,12,13	10,12
TPOX	8,10	8,10	7,8,10	8,10
DY3S91	INC	---	INC	---
D12S301	12,13,15	12,15	12,13,15	12,15
D19S433	15,17	15,17	15,17	15,17
FGA	20,23,26	20,23	20,23,26	20,23
D22S1048	15	15	15	15

iSWAB-DNA Collected Samples are Stable Over 5 Years at Room Temperature



45°C	Day 1	Day 3	Day 7	Day 12	Day 18	Day 27	Day 36	Day 45	Day 60	Day 90	Day 180
Ref	10	10	10	10	10	10	10	10	10	10	10
ND	10	10	10	10	10	10	10	10	10	10	10

5. Summary and Conclusions

- iSWAB-ID efficiently recovered and stabilized DNA of forensic significance at the point of collection.
- iSWAB-ID stabilized DNA remained of sufficient quality to analyze for at least 2 weeks at ambient temperature.
- Unlike processing swabs, collecting samples with iSWAB-ID allows for multiple runs for analysis and archiving purposes
- Accelerated stability testing of iSWAB-ID collected DNA suggest >5 year stability at room temperature.
- iSWAB-ID lysis and DNA release from collected material increases with time.
- AnaPrep DNA extractor, using the blood DNA kit, was compatible with iSWAB-ID
- DNA of low to high copy, stored in iSWAB-ID, could be efficiently purified by AnaPrep
- AnaPrep-extracted DNA performed well with industry standard Forensics DNA tests
- Proper recovery and stabilization of DNA samples of forensic significance with iSWAB-ID is critical for improving the STR profiling call rates, and especially important for maintaining the integrity of the collected sample during protracted transit or processing backlogs**