

# iSWAB-DNA: Ensuring Long-Term Stability of Genomic Samples for Reliable Downstream Analysis.

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## Introduction

The collection of non-invasive samples, such as buccal cells swabs and saliva samples, were significant approaches in the field of genomic testing for diagnostic purposes and personal exploration of health and lifestyle optimization. Biobanking of these samples creates opportunities for new research, resolution of numerous unanswered questions. However, the economic sustainability of biobanks has emerged as a major concern. The iSWAB-DNA device from Mawi DNA Technologies presents a long-term storage method under ambient temperature to make biobanking more economic sustainable. With its innovative non-toxic stabilization buffer, the iSWAB-DNA device blocks prokaryotic cells, slowly lyses eukaryotic cells, and stabilizes the integrity of the DNA at ambient temperatures for long term. In this study, the iSWAB-DNA device was tested for long-term stability with real-time aging and accelerate aging.

## Methods

### Samples collections:

Buccal swabs samples were collected in iSWAB-DNA devices from ten donors according to the respective standard instructions for use (IFU). All collected samples were mixed at 12 rpm for 24 hours to simulate movement of transportation. After baseline (day 1) assessment, all samples were stored at ambient temperature (24°C) or at 56 °C for long term storage.

### DNA extraction:

#### Long-term storage at 24°C for a year:

Twenty-one samples collected in iSWAB-DNA-1200 devices from ten donors were stored at ambient temperature (24°C) for up to a year. DNA from all samples were extracted using QIAamp Blood DNA Kit (Qiagen, Cat #51185) at baseline, 30 days, 60 days, and one year. DNA yield was compared between 4 time points. DNA integrity was compared between baseline and one year.

#### Accelerate Aging – long-term storage at 56°C for 117 days:

To forecast stability, accelerated testing is frequently utilized. It is based on the Arrhenius equation, which states that the rate of a chemical reaction doubles for every 10°C rise in temperature. In our study, ten samples collected in iSWAB-DNA-1200 devices from ten donors were stored at 56 °C for 117 days, which is equivalent to 3 years of storage at room temperature. DNA was extracted using QIAamp Blood DNA Kit (Qiagen, Cat #51185) at days 0, 3, 6, 12, 17, 26, 34, 43, 117 corresponding to anticipated age of 0, 1, 3, 4, 6, 9, 12, 15, 36 months for room temperature stored samples. DNA yield, purity, and integrity were compared between samples with different anticipated ages.

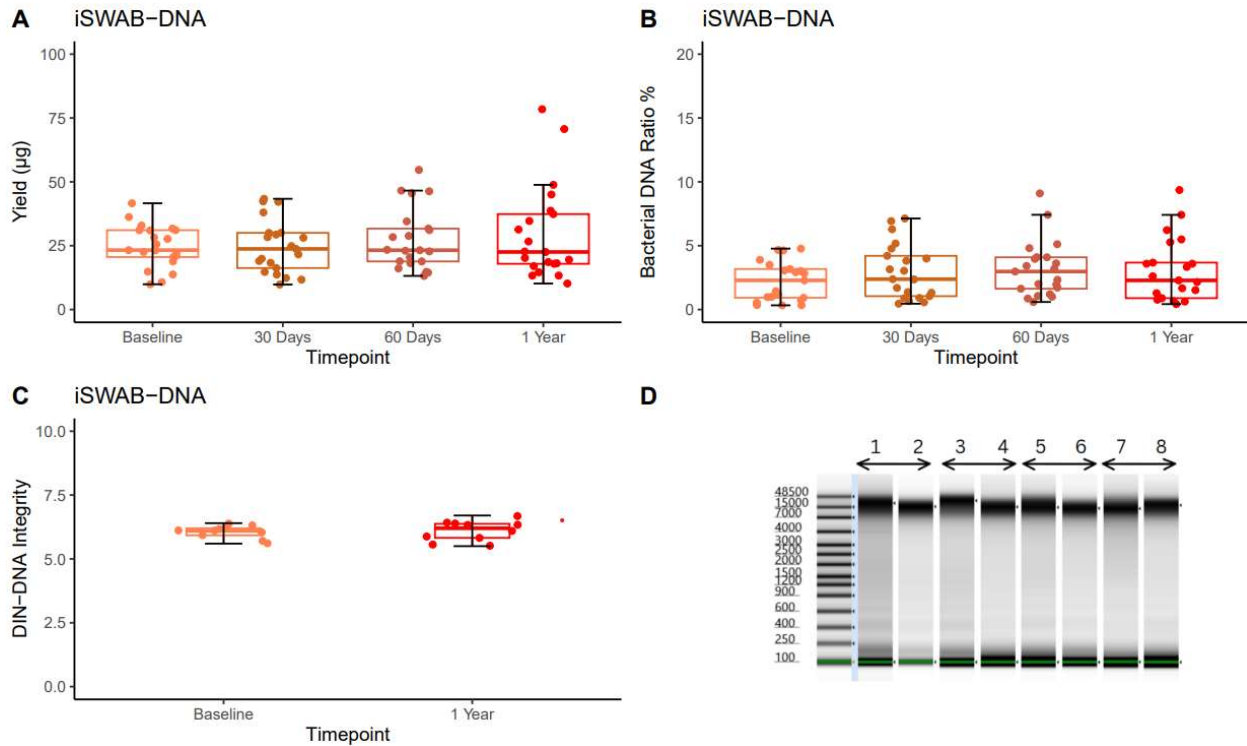
### DNA quantification and qualification:

DNA quantity and integrity was measured using Nanodrop One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific Cat. # ND-ONE-W4) and TapeStation 4150 System with Agilent Genomic DNA

ScreenTapes (Agilent, Cat. # 5067-5365). Bacterial DNA ratio was assessed with qPCR targeting 16S rRNA gene. Detailed protocols will be provided upon request.

## Results and Discussion

Long-term storage at 24°C for a year:



**Figure 1: iSWAB-DNA devices stabilized DNA for up to one year.** A, DNA yield was calculated based on the volume of devices (iSWAB-DNA-1200). B, Bacterial DNA ratio at each time point. C, DNA Integrity. D, Gel image (Tape Station system) of purified DNA extracted from the same 4 samples at baseline (1, 3, 5, 7) and 1 year (2, 4, 6, 8).

To determine the real-time stability of iSWAB-DNA devices, samples were collected and stored at ambient temperatures for one year. No significant changes on DNA yield, integrity, and bacterial DNA ratio were observed after one year of storage (Figure 4A-C). Additionally, the gel displays purified DNA with high molecular weight for the one-year-old samples, indicating the successful preservation of high molecular weight DNA (Figure 4D). Above all, DNA from samples collected in iSWAB-DNA devices remain stable after one year of ambient temperature storage. Further testing of these post-collection samples in iSWAB-DNA devices is ongoing to determine the optimal long-term stability.

Accelerate Aging – long-term storage at 56°C for 117 days:

Accelerated-aging studies were commonly used for testing shelf life of devices with concurrent real-time studies. It offers valuable insights into stability, providing projected information with time efficiency and cost-effectiveness. To examine the optimal stability of the post-collection samples in iSWAB-DNA devices, an accelerated-aging study was conducted. Samples collected in iSWAB-DNA devices were stored at 56°C for 117 days corresponding to 36 months at ambient temperature (24°C) in real time. During the accelerated testing, there was no significant alteration observed in the DNA yield, DNA purity and bacterial levels of all the samples at all time points (Figure 3A-D). DNA integrity (DIN) displayed a minor decrease trend at the final time point (36 months) without being significant (Figure 3C). Bacterial DNA ratio displayed a minor increase trend without being significant (Figure 3D). Nonetheless, the purified DNA still exhibited a condensed DNA band at high molecular weight without smear (Figure 3E), which is suitable for many downstream applications. Therefore, based on the results of the accelerated stability testing, samples collected using iSWAB-DNA devices could be forecasted to maintain their stability for up to 3 years at ambient temperatures (56°C for 117 days).

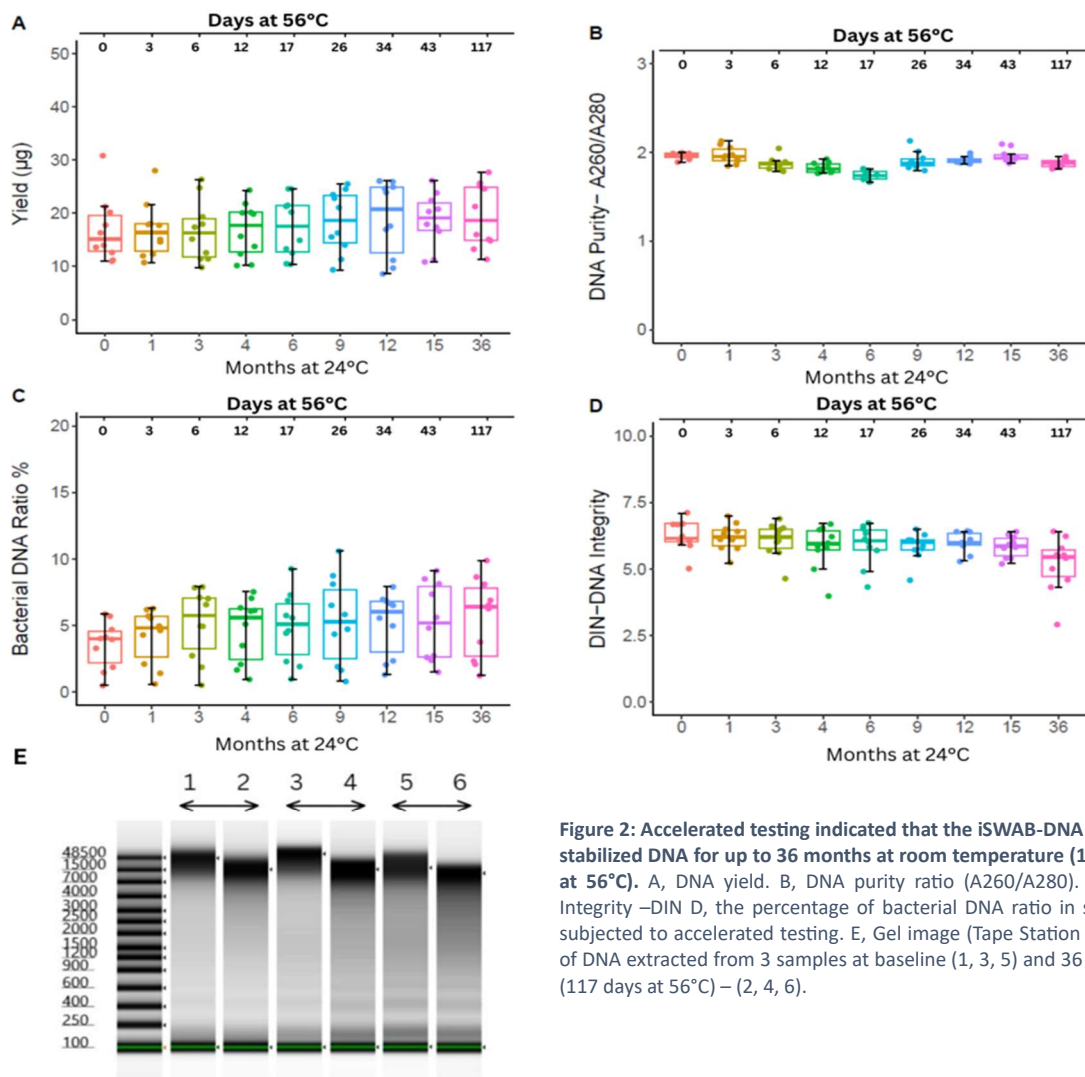


Figure 2: Accelerated testing indicated that the iSWAB-DNA devices stabilized DNA for up to 36 months at room temperature (117 days at 56°C). A, DNA yield. B, DNA purity ratio (A260/A280). C, DNA Integrity –DIN D, the percentage of bacterial DNA ratio in samples subjected to accelerated testing. E, Gel image (Tape Station system) of DNA extracted from 3 samples at baseline (1, 3, 5) and 36 months (117 days at 56°C) – (2, 4, 6).

## Conclusion

iSWAB-DNA devices developed by Mawi DNA Technologies have exhibited the capability for long-term DNA preservation up to 3 years. Real-time testing has shown preservation for up to 12 months at ambient temperature. Accelerated testing has demonstrated the iSWAB-DNA devices' ability to maintain sample quality at ambient temperature for up to 36 months, and at temperature as high as 56°C for at least 117 days. Among all the time points tested, samples in iSWAB-DNA devices yield high quality and quantity DNA suitable for diverse downstream applications. Also, proprietary stabilization buffer utilized in the iSWAB-DNA devices has proven advantageous in preserving the relative abundance of bacterial species even after prolonged storage periods. All above advantage features will contribute to enhanced efficiency and cost-effectiveness of downstream applications, making iSWAB-DNA a valuable tool in scientific research and diagnostics.