Technical Note

Evaluation of Performance of gDNA Extraction from CollectEject™ Swabs and PCR/Sanger Sequencing Analysis; a Comparison with Leading Swab Device

Introduction

With over 30 years of experience, Gentueri specializes in developing and manufacturing non-invasive sample collection devices for biomedical research and forensics applications. Gentueri's CollectEject™ Oral Swabs are ideal for easy and convenient collection of oral/buccal samples in a clinic office or a remote setting. The multi-layer cellulose swab head provides maximum sample absorption and releases for high DNA yield and quality.

The purpose of this study is to assess the performance of the CollectEject Oral swabs using the Promega Maxwell 16 Buccal Swab Kit for DNA isolation followed by PCR and then Sanger sequencing of the PCR products. Extractions were performed at various time points after sample collection to determine if changes in DNA quality and integrity occurred, and head-to-head comparisons were made with a leading competitive oral sample collection kit. Data is presented on (a) success rates for quality, quantity, and integrity of isolated DNA, and (b) Sanger Sequencing success rates on two target gene (BMP-15) exons.

All extraction protocols, PCR experiments, and Sanger Sequencing reactions were performed by a CLIA-and CAP-certified independent laboratory using a clinically validated PCR/Sanger assay for BMP – 15 gene. The CollectEject oral swabs with collected samples were placed directly into the validated DNA extraction procedure without optimizing lysis steps or extraction protocols.

Materials and Methods

Sample Collection and Preparation

Forty buccal swabs were collected from consenting adult volunteers using the CollectEject Oral swabs and forty swabs with collected samples from a leading competitor (Competitor A).

CollectEject swabs were collected for 20 seconds under the tongue and then rubbed the inside of both cheeks for 20 seconds each (one minute total time). Competitor A samples were collected and stored according to the manufacturer's instructions. Collected CollectEject samples were stored in Gentueri GenDry™ pouches with desiccant to dry and preserve the sample at ambient temperature.

The time of collection to extraction schedule was 1 day, 2 days, 3 days, 7 days, and 14 days. Batches of collected samples were shipped at room temperature at specified time points to the independent lab, and samples were processed the same day they were received.

Maxwell 16 Buccal Swab Kit and Extraction Genomic DNA were isolated from buccal swab samples using Maxwell 16 Buccal Swab LEV DNA Purification Kit and the Maxwell 16 MDx instrument (Promega) according to the manufacturer's protocols.

Assessment of DNA Quality, Quantitation, and Integrity

A NanoDrop ND-8000 UV/VIS spectrophotometer (ThermoFisher) was used according to the manufacturer's instruction manual to determine DNA quality. The DNA quality success benchmark was OD 260/280 ratio ≥ 1.4.

A Qubit® dsDNA assay (ThermoFisher) and DeNovix QFX fluorometer (DeNovix Inc.) were used according to the manufacturer's instructions to determine DNA quantity. The DNA quantity success benchmark was total DNA ≥ 500 ng.

To determine DNA integrity, isolated gDNA was analyzed by agarose gel electrophoresis. Only gDNA bands that were clearly detectable were used in this study. Five (5) ul of gDNA and 1kb DNA ladder were checked on 0.8% agarose gel.

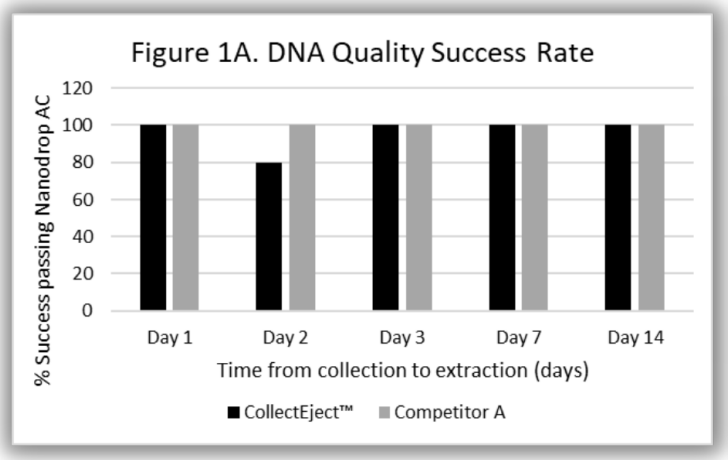
PCR and Sanger Sequencing

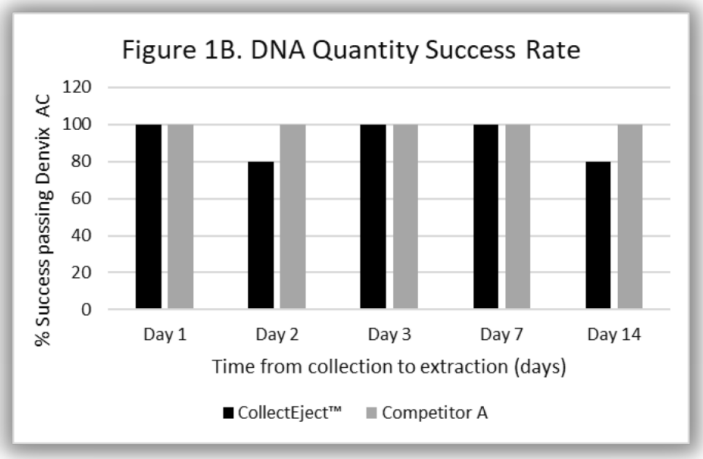
Two target gene (BMP 15) exons were PCR amplified from each sample's genomic DNA using KAPA 2G Robust Hot Start PCR Kit (KAPA BioSystems Inc.).

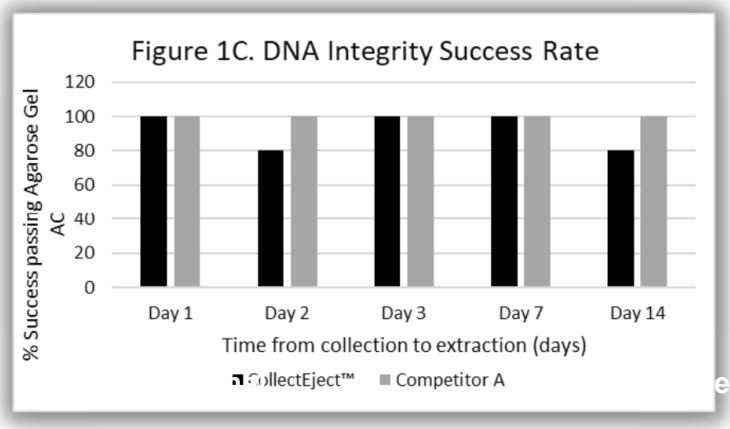
PCR products were sequenced using PCR primers and internal sequencing primers with a standard reaction setup and with BigDye™ v 3.1 Terminator Cycle Chemistry Kit (ThermoFisher). Primer extension reactions were analyzed on a 3730 xl DNA Analyzer (ThermoFisher).

Only exon target reads with at least 2-fold coverage and with PHRED 20 or higher base call quality was used for analysis.

Results and Conclusions







Figures 1A, 1B, and 1C.Success rates for DNA quality, quantity, and integrity for both devices are shown. Both devices performed well across all time points.

The optimized, validated assay using Competitor A device performed only slightly better than the CollectEject swab.

Ph: +1-608-949-8568
Email: info@gentueri.com
Website: www.gentueri.com

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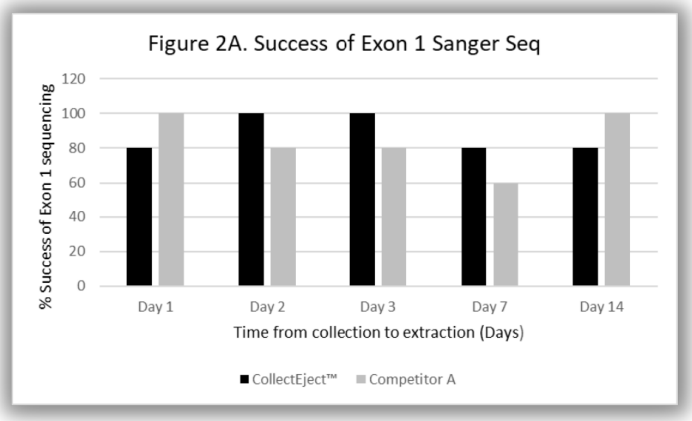

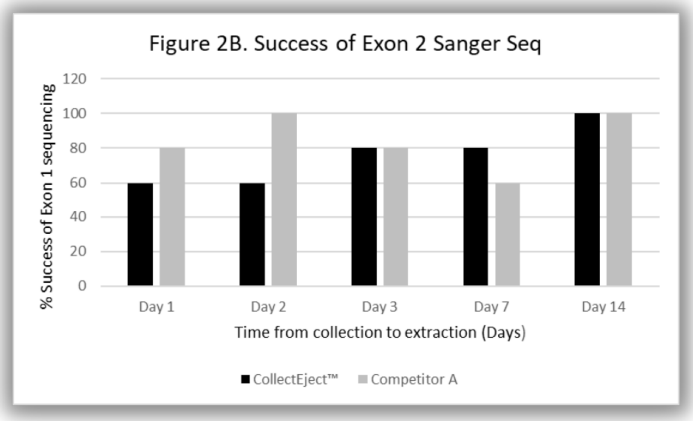

Figures 2A and 2B. The success rates for CollectEject Oral Swab and Competitor A are compared for the two BMP 15 exons (1 and 2). Both devices worked well for both exons.

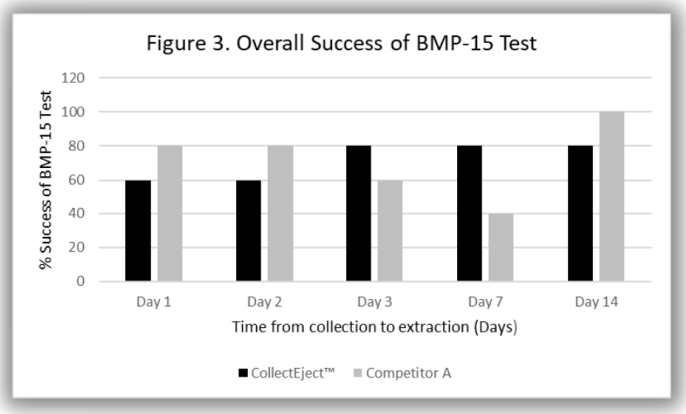

Figure 3. The overall success rate for the BMP-15 test was very similar for the CollectEject Oral Swab and Competitor A.

There was no discernable effect of testing on different days after sample collection. Both devices had a combined success rate of 72%.

Summary

Using a clinically validated assay for human BMP 15 gene and performed by a CLIA- and CAP-certified independent laboratory, the Gentueri CollectEject Oral swab was compared to a competitor's buccal collection device. Test results for DNA quality, DNA quantity, and DNA integrity indicate similar success characteristics across various times from sample collection to DNA extraction. Combined overall success rates for the BMP-15 test using both collection swab types were identical at 72%.

Ph: +1-608-949-8568
Email: info@gentueri.com
Website: www.gentueri.com

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