
Technical Note

PAXgene[®] Saliva Collector Robustness Studies for DNA Applications

The objective of these studies was to test the robustness of the PAXgene Saliva Collector. In the studies, the preanalytical steps, including handling, storage and processing, were conducted according to Technical Specification CEN/TS 17305:2019 (Molecular in vitro diagnostic examinations – Specifications for pre-examination processes for saliva – Isolated human DNA).

Introduction

Saliva is increasingly used as a non-invasive alternative specimen to blood for the examination of human DNA. The PAXgene Saliva Collector is part of a comprehensive pre-analytical workflow for human saliva collection. The workflow includes stabilization, transport and storage, progressing through DNA extraction and analysis. The collection device itself contains a stabilizing solution which stabilizes the DNA levels in human saliva samples by protecting DNA from degradation and inhibiting bacterial growth over storage time and is compatible to existing QIAGEN[®] manual and automated extraction solutions (**Figure 1**).

PreAnalytiX[®] developed the PAXgene Saliva workflow in order to minimize post-collection changes caused by preanalytical variables and to standardize the preanalytical steps from saliva collection until nucleic acid is available for molecular analyses.

PAXgene Saliva Workflow

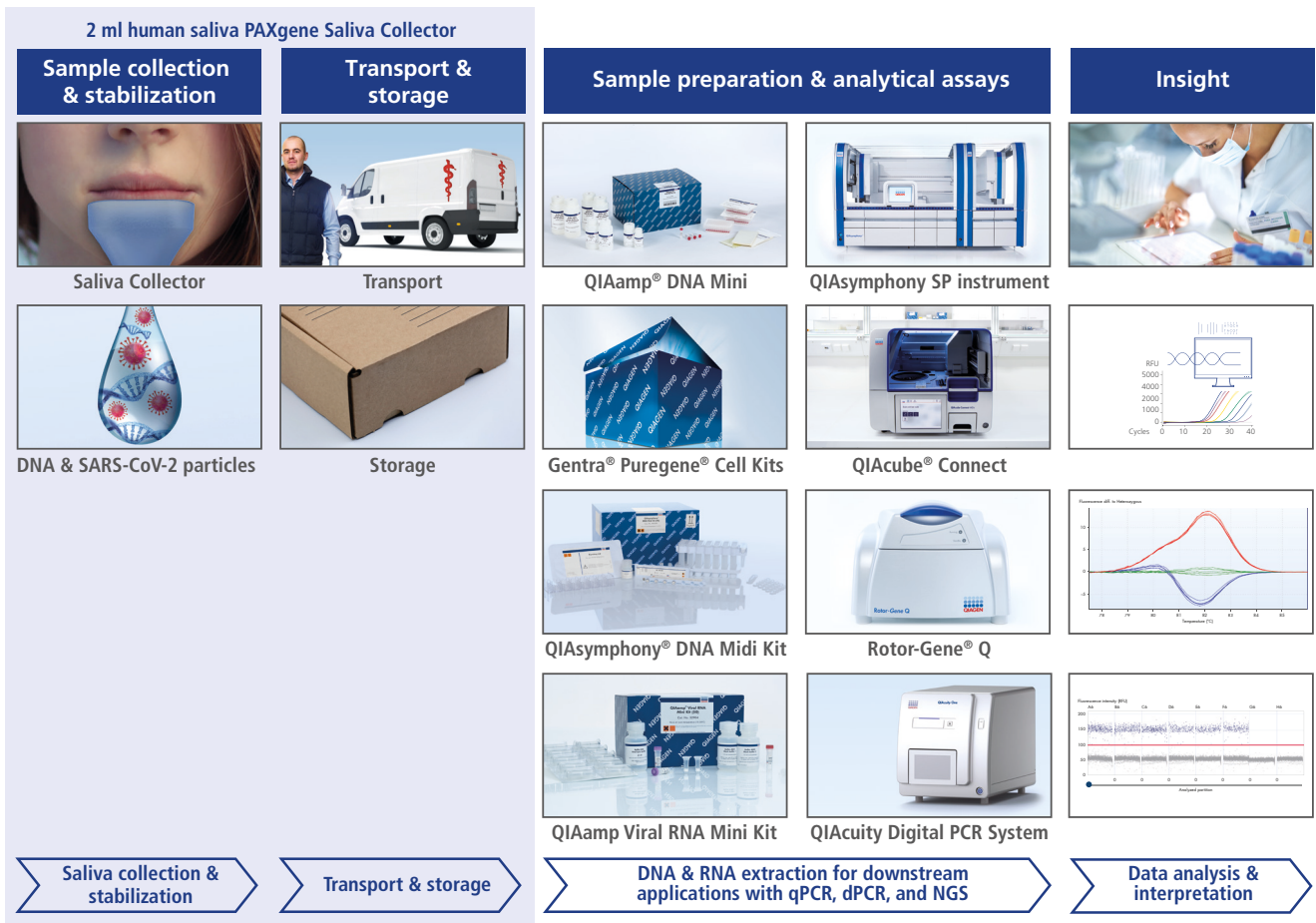


Figure 1. PAXgene Saliva Workflow*

Saliva collected into PAXgene Saliva Collector can be used to process DNA automated with the QIAasyphony® DNA Midi Kit on the QIAasyphony SP Instrument or the QIAamp® DNA Mini Kit on the QIAcube (Classic and Connect). Manual extraction can be performed with the QIAamp DNA Mini and Gentra® Puregene® Cell Kit. SARS-CoV-2 RNA can be isolated with the QIAamp Viral RNA Mini Kit. Nucleic acids can be used for molecular test methods including qPCR, dPCR and NGS.

* For molecular biology applications. Not intended for the diagnosis, prevention, or treatment of a disease.

The PAXgene Saliva Collector is a plastic device consisting of a collection funnel attached to a collection tube. According to the PAXgene Saliva Collector instructions for use, saliva is spit into the funnel until it reaches a fill line indicating a 2 ml filling volume. When the fill line is reached, the funnel is unscrewed from the tube to release a stabilizing solution. After a lid is placed over the opening of the tube and screwed tightly, the tube is inverted 5 times to mix saliva and stabilizing solution (see PAXgene Saliva Collector Instructions for use, is available at www.preanalytix.com).

However, users might collect more or less saliva than indicated by failing to notice the fill line or underestimating amount of bubbles in the primary sample. They might invert the tube fewer times than required. Also, saliva samples might contain elevated levels of potentially interfering endogenous substances.

In this technical note, we present the results from robustness studies designed to determine how inappropriate filling and mixing as well as elevated levels of endogenous substances influence the ability of the PAXgene Saliva Collector to stabilize DNA yield and purity in human saliva.

Study Design

Individual primary saliva samples were collected from consented, apparently healthy adult subjects. These were internal voluntary donations at QIAGEN (Hilden). Subjects followed written instructions provided to them. Donors were asked not to eat, drink, chew chewing gum or smoke 30 minutes prior to donation.

In order to achieve a volume of saliva large enough for different test conditions, saliva samples from different donors were pooled. Saliva was collected in 15 ml conical tubes and pooled by pipetting directly after collection. The pooled saliva was stirred with a magnetic stirrer and resuspended by pipetting up and down for at least 20 minutes to assure sufficient homogenization.

After distribution into single PAXgene Saliva Collectors, 200 µl samples were processed manually with the QIAGEN QIAamp DNA Mini kit using the spin protocol with 50 µl elution. Eluates were analysed for purity (A_{260}/A_{280} ratio) by spectrophotometric analysis on a NanoDrop™ Spectrophotometer (Thermo Fisher Scientific) and for DNA yield, and PCR performance with a quantitative qPCR assay, the QIAGEN Investigator® Quantiplex® Pro RGQ Kit on the QIAGEN Rotor-Gene® Q. Absolute quantification was performed according to the Investigator kit handbook by comparing the CT values from individual samples to a linear regression line generated from a series dilution of the absolute quantification standard for human target provided in the kit.

Conditions tested

Study 1: Underfilling/Overfilling

Saliva was collected from 24 donors using 15 ml conical tubes. Each donor provided 3.5 ml of saliva. Directly following collection, all saliva was pooled into groups of three donors to create eight pools of 10.5 ml each. Different volumes of saliva from each pool were distributed by pipetting onto five different PAXgene Saliva Collectors per pool and mixed with the stabilizing solution by inverting the tube 5 times (**Table 1**).

Filling Condition	100% (Reference)	30%	70%	120%	140%
Saliva volume [ml]	2.0	0.6	1.4	2.4	2.8
Pool	1–8	1–8	1–8	1–8	1–8
PAXgene Saliva Collector	1-8	9-16	17-24	25-32	33-40

Table 1. PAXgene Saliva Collector filling conditions for Underfilling/Overfilling Robustness Study

From each PAXgene Saliva Collector, one aliquot was processed within 2 hours of filling and one after storage for 30 days at room temperature.

Study 2: Inappropriate Mixing

Saliva was collected from 24 donors using 15 ml conical tubes. Each donor provided 3.5 ml of saliva. Directly after collection, all saliva was pooled into groups of four donors, creating six pools of saliva containing 14 ml each. Using a pipette, 2 ml of saliva from each pool was distributed onto six different PAXgene Saliva Collectors with duplicates per tested condition. Three different conditions for mixing saliva and stabilizing solution were tested: 5 times mixing (reference), 2 times and 0 times mixing (both of which are inappropriate, see **Table 2**).

Saliva with stabilizing solution Mixing Condition	5x inverted (Reference)	0 inverted	2x inverted
Saliva volume [ml]	2.0	2.0	2.0
Pool	1–6	1–6	1–6
PAXgene Saliva Collector	1–12	13–24	25–36

Table 2. PAXgene Saliva Collector conditions for Inappropriate Mixing Robustness Study

From each PAXgene Saliva Collector, one aliquot was processed within 2 hours of filling and one after storage for 14 days at room temperature.

Study 3: Endogenous Interfering Substances

Saliva was collected from 30 donors in 15 ml conical tubes. Each donor provided 4 ml of saliva. Following collection, saliva was pooled in groups of three donors to create 10 pools of 12 ml of saliva. Using a pipette, 2 ml of saliva from each pool was distributed onto six different PAXgene Saliva Collectors. The Saliva Collectors were then inverted 5 times to mix saliva and stabilizing solution. Endogenous substances were spiked in directly after mixing (**Table 3**). As reference, two PAXgene Saliva Collectors from each pool were left un-spiked.

Added endogenous Interfering Substance	Reference no spike in	Albumin	D-(+) Glucose	Hemoglobin
Saliva volume [ml]	2.0	2.0	2.0	2
Pool	1–10	1–10	1–10	1–10
PAXgene Saliva Collector	1–20	21–30	31–40	51–60
Spike in to 2 ml Saliva (per device)	–	12 mg	1 mg	6 µg

Table 3. PAXgene Saliva Collector spike-in of endogenous interfering substances.

From each PAXgene Saliva Collector, one aliquot was processed within 2 hours of filling and one after storage for 14 days at room temperature.

Results

Study 1: Underfilling/Overfilling

According to the instructions for use, the PAXgene Saliva Collector needs to be filled with 2 ml saliva. To simulate underfilling or overfilling, between 0.6 to 2.8 ml saliva from saliva pools were pipetted into single devices (**Table 1**) and saliva was processed within 2 hours (d_0) or after storage for 30 days (d_{30}) at room temperature.

The investigated range of underfilling and overfilling had no or minor effects on DNA yield per ml saliva. Also, purity was consistently in the range of 1.6–2.0 with one considerable outlier in case of 30% filling and storage for 30 days, the most stringent condition tested (**Figure 2**).

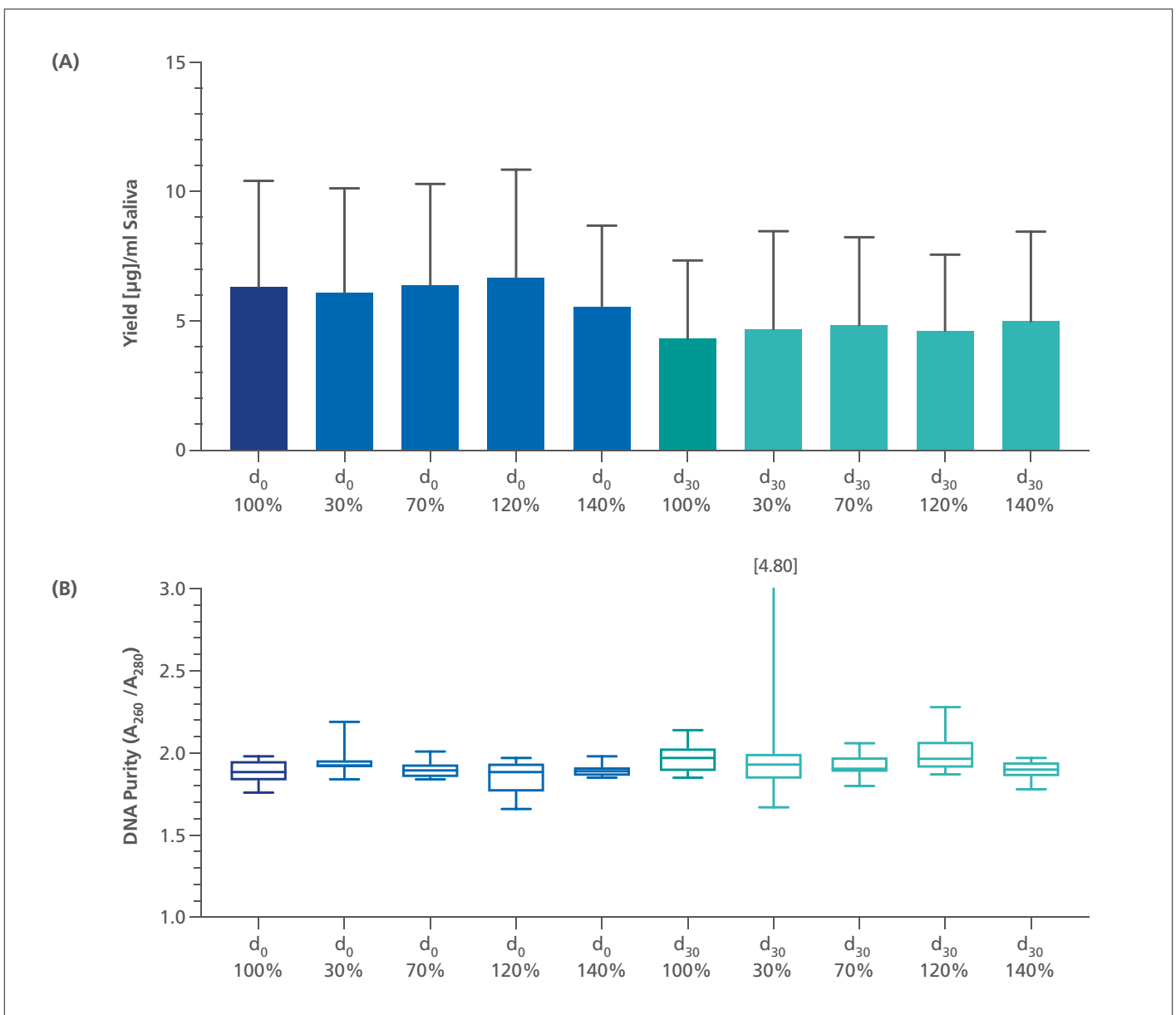


Figure 2. Impact of PAXgene Saliva Collector underfilling/overfilling on DNA yield and purity.

Saliva stabilized in correctly filled (100%), underfilled (30% and 70%) or overfilled (120% and 140%) PAXgene Saliva Collector samples were processed within 2 hours (d_0) and after 30 days storage at room temperature (d_{30}). DNA from stabilized saliva was extracted with the QIAamp DNA Mini kit and analyzed for purity (A_{260}/A_{280} ratio) by spectrophotometric analysis and for DNA yield by qPCR with the Investigator Quantiplex Pro RGQ Kit on the Rotor-Gene Q.

(A) DNA yield per ml saliva; values are means with standard deviation, $n = 8$.

(B) DNA purity; box plots with medians, 25th and 75th percentiles and minimum to maximum, $n = 8$.

Study 2: Inappropriate Mixing

In order to efficiently mix stabilizing solution and saliva, the PAXgene Saliva Collector instructions for use state to gently invert the tube 5 times after collection. To test the impact of inappropriate handling, aliquots from saliva pools after transfer into PAXgene Saliva Collector were mixed correctly 5 times (reference) or inappropriately 0 or 2 times (**Table 2**).

The investigated inappropriate mixing conditions had no or a minor effect on DNA yield and purity when saliva was processed within 2 hours after distributing to PAXgene Saliva Collector (d_0) or after storage for 14 days (d_{14}) at room temperature (**Figure 3**).

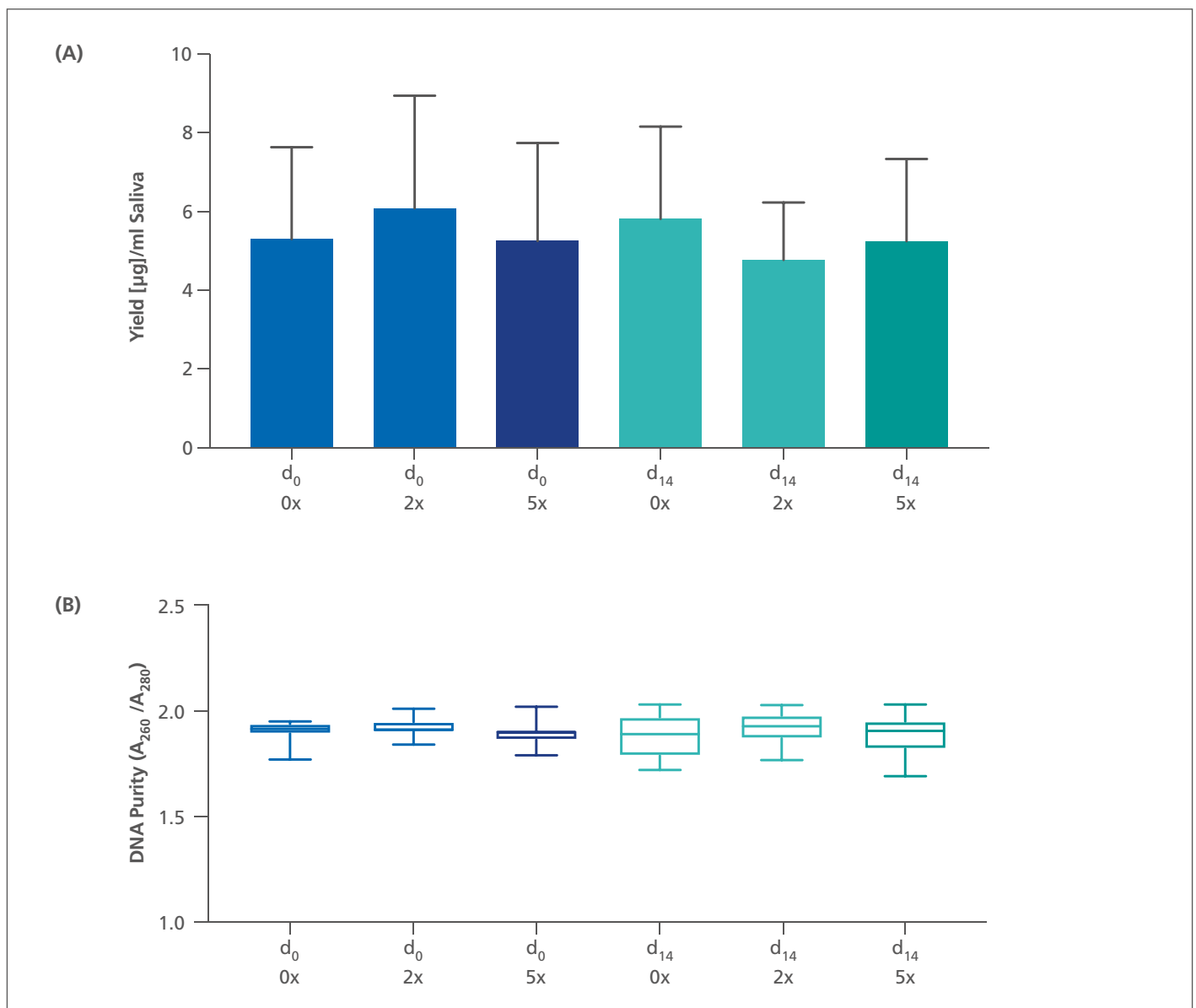


Figure 3. Impact of inappropriate mixing on DNA yield and purity.

Saliva stabilized in a correctly-filled PAXgene Saliva Collector was mixed 5 times according to instructions for use, or inappropriately without inversion or only inverted twice. Saliva was processed within 2 hours (d_0) and after 14 days storage at room temperature (d_{14}). DNA from stabilized saliva was extracted with the QIAamp DNA Mini kit and analysed for purity (A_{260}/A_{280} ratio) by spectrophotometric analysis and for DNA yield by qPCR with the Investigator Quantiplex Pro RGQ Kit on the Rotor-Gene Q.

(A) DNA yield per ml saliva; values are means with standard deviation, $n = 12$.

(B) DNA purity; box plots with medians, 25th and 75th percentiles and minimum to maximum, $n = 12$.

Study 3: Endogenous Interfering Substances

To verify that elevated levels of endogenous substances in saliva do not interfere with DNA extraction, quality and functional performance, aliquots of 10 saliva pools were distributed into PAXgene Saliva Collectors and spiked with potentially interfering substances (**Table 3**).

Substances investigated had no or a minor impact on DNA yield when saliva was processed within 2 hours after distributing into PAXgene Saliva Collector and spike-in (d_0) or after storage for 14 days at room temperature (d_{14}) (**Figure 4**).

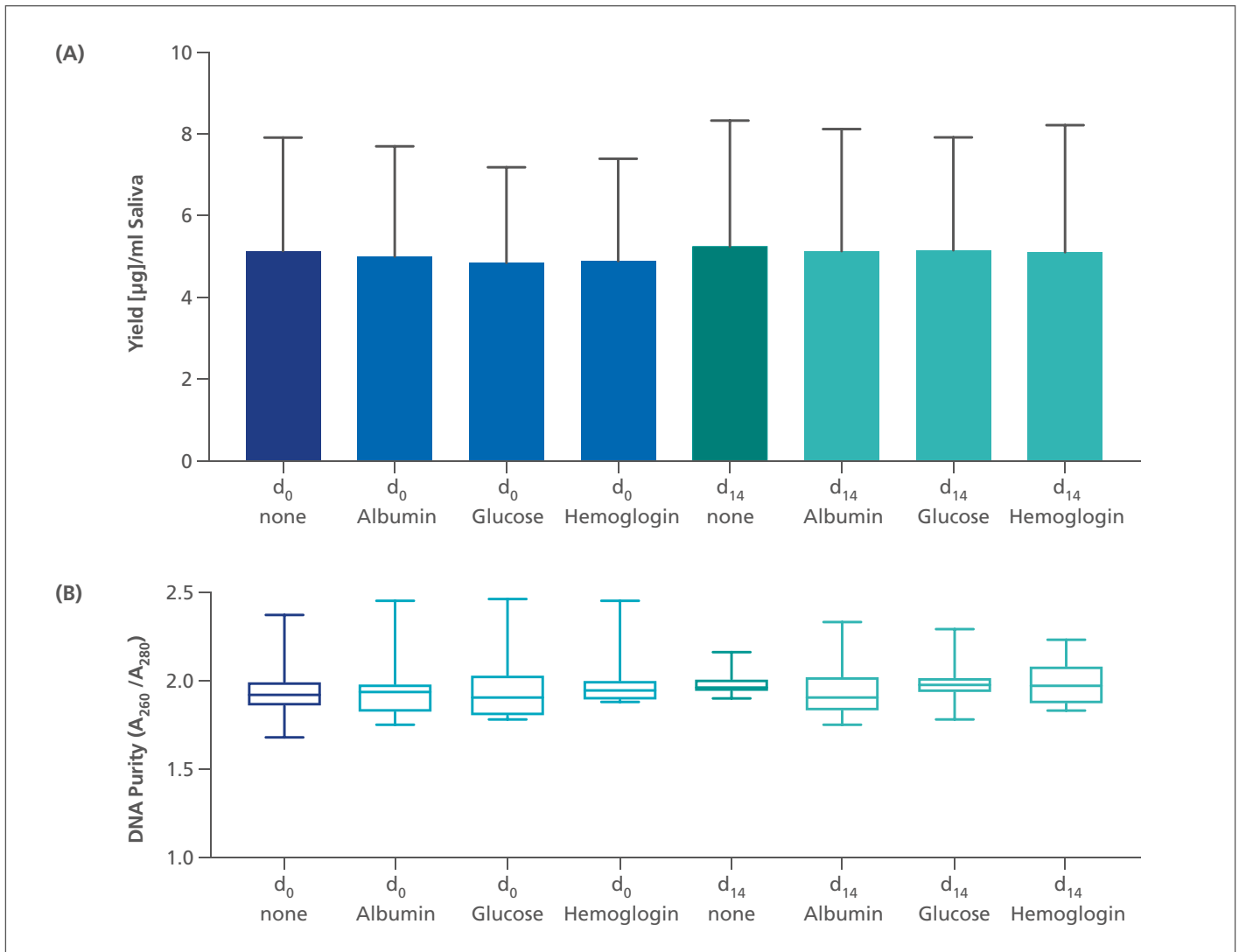


Figure 4. Impact of endogenous potentially interfering substances on DNA yield and purity.

Saliva stabilized in correctly filled and inverted PAXgene Saliva Collectors was spiked with interfering substances (**Table 3**). Saliva was processed within 2 h (d_0) and after 14 days storage at room temperature (d_{14}). DNA from stabilized saliva was extracted with the QIAamp DNA Mini kit and analyzed for purity (A_{260}/A_{280} ratio) by spectrophotometric analysis and for DNA yield by qPCR with the Investigator Quantiplex Pro RGQ Kit on the Rotor-Gene Q.

(A) DNA yield per ml saliva; values are means with standard deviation, $n = 10$.

(B) DNA purity; box plots with medians, 25th and 75th percentiles and minimum to maximum, $n = 10$.

Conclusion

In the studies presented here, the impact of inappropriate handling of the PAXgene Saliva Collector, such as underfilling and overfilling, or incomplete mixing of saliva with stabilizing solution, as well as elevated level of endogenous substances in saliva have no or a minor impact on the ability of the PAXgene Saliva Collector to stabilize DNA yield and purity in human saliva.

Overall, the study results demonstrate robustness of the PAXgene Saliva Collector workflow. In contrast to invasive specimen collection, saliva collection with the PAXgene Saliva Collector does not require trained and educated professionals or dedicated facilities to collect the saliva sample. With the instructions for use provided with each collection device, the workflow is robust and easy to use for unassisted, self-collection at home or out of doctor's office, for most people including elderly and young donors.

Although robustness was demonstrated, for optimal results it is recommended to follow the instructions within the PAXgene Saliva Collector Instructions for Use.

Products used

Product	Catalog No.
PAXgene Saliva Collectors (25)	769040
QIAamp DNA Mini Kit (50)	51304
Investigator Quantiplex Pro RGQ Kit (200)	387316

All products used in this technical note are intended for molecular biology applications. They are not intended for the diagnosis, prevention, or treatment of a disease.



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