Title
Disposable collection kit for rapid and reliable collection of saliva.

Permalink
https://escholarship.org/uc/item/9d49r09f

Journal
American journal of human biology : the official journal of the Human Biology Council, 27(5)

ISSN
1042-0533

Authors
Yamaguchi, Masaki
Tezuka, Yuki
Takeda, Kazunori
et al.

Publication Date
2015-09-01

DOI
10.1002/ajhb.22696

Peer reviewed
Disposable Collection Kit for Rapid and Reliable Collection of Saliva

MASAKI YAMAGUCHI,1 YUKI TEZUKA,1 KAZUNORI TAKEDA,2 AND VIVEK SHETTY3

1Biomedical Engineering & Robotics Laboratory, Graduate School of Engineering, Iwate University, 4-3-5 Ueda, Morioka 020-8551, Japan
2Graduate Course of Disability Science, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8577, Japan
3Section of Oral and Maxillofacial Surgery, UCLA School of Dentistry, University of California, 10833 Le Conte Avenue, Los Angeles, California 90095-1668

Objectives: To describe and evaluate disposable salivary collection kit for rapid, reliable, and reproducible collection of saliva samples.

Methods: The saliva collection kit comprised of a saliva absorbent swab and an extractor was used to retrieve whole saliva samples from 10 subjects. The accuracy and precision of the extracted volumes (3, 10, and 30 µl) were compared to similar volumes drawn from control samples obtained by passive drool. Additionally, the impact of kit collection method on subsequent immunoassay results was verified by assessing salivary cortisol levels in the samples and comparing them to controls.

Results: The recovered volumes for the whole saliva samples were 3.85 ± 0.28, 10.79 ± 0.95, and 31.18 ± 1.72 µl, respectively (CV = 8.76%) and 2.91 ± 0.19, 9.75 ± 0.43, and 29.64 ± 0.91 µl, respectively (CV = 6.36%) for the controls. There was a close correspondence between the salivary cortisol levels from the saliva samples obtained by the collection kit and the controls ($R^2 > 0.96$).

Conclusions: The disposable saliva collection kit allows accurate and repeatable collection of fixed amounts of whole saliva and does not interfere with subsequent measurements of salivary cortisol. The simple collection process, lack of elaborate specimen recovery steps, and the short turnaround time (<3 min) should render the kit attractive to test subjects and researchers alike. Am. J. Hum. Biol. 27:720–723, 2015. © 2015 The Authors American Journal of Human Biology Published by Wiley Periodicals, Inc.
commonly used biomarker of the stress response, to verify the impact of the collection method on subsequent immunoassay results.

MATERIALS AND METHODS

Saliva collection kit

The disposable saliva collection kit is comprised of a saliva absorbent swab and an extractor unit (Fig. 1A, B). The absorbent swab (20 mm length x 3.5 mm diameter) consists of a sponge-like braided core of polypropylene resin foam (fiber density of 0.09 g/cc) contained within a polyethylene sleeve. The saliva extractor consists of a polypropylene barrel for receiving the absorbent swab and a corresponding plunger for squeezing out the contained saliva. The distal end of the barrel opens into a narrower reservoir that stores the expressed saliva. The reservoir empties into a sample tube whose inner surface is treated with a surfactant using sucrose esters of fatty acids (DK Ester S-L18A, Dai-Ichi Kogyo Seiyaku Co., Ltd., Japan). Printed graduation marks on the sample tube allow saliva volumes to be easily verified. A contiguous aspirator permits controlled withdrawal of saliva from the reservoir into the sample tube.

To obtain a saliva specimen nonstimulated whole saliva is allowed to pool at the floor of the mouth for approximately 1 min. The subject is instructed to place the absorbent swab under the tongue for another minute (Fig. 1C-i). The constant dimensions and microstructure of the resin foam ensures that the absorbent swab saturates with consistent quantities (~100 µl) of saliva within a minute and is independent of the salivary flow rates. The saturated swab is retrieved by expressing swab directly into the barrel of saliva extractor. The plunger is inserted and pushed down to express the contained saliva into the reservoir (Fig. 1C-ii). Next, the aspirator is activated to draw saliva held within the reservoir into the sample tube until the level reaches the graduation mark corresponding to the desired volume (Fig. 1C-iii). The constant inner diameter of the sample tube (0.79 mm) ensures that the aspirate volume is replicable and precise. Finally, the sample tube is disconnected from the reservoir and the aspirate plunger pushed to discharge a precise amount of saliva for assay purposes (Fig. 1C-iv).

Verification of saliva volume recovery

The saliva collection kit was used to obtain whole saliva samples from 10 consenting, healthy adult subjects (22.6 years ± 1.3 year) using procedures approved by the Institutional Review Board. Concomitantly, we also used the collection kit to collect corresponding volumes (n = 10)
from standard cortisol solution included in a commercialized ELISA (enzyme-linked immune sorbent assay) kit (control sample). The volumes of collected samples (saliva and cortisol standard) were verified by weighing recovered samples on an electronic balance (10 mg resolution, GR-202, A&D Co., Ltd, Japan) and then converting measured weights into corresponding volumes.

**Impact on measured levels of salivary cortisol**

To determine the impact of absorbent swab on the measurement of salivary cortisol (sCortisol) levels, we compared calibration curves for cortisol concentration obtained from whole saliva samples from the saliva collection kit and the micropipetting approach. A passive drool saliva sample (~3 ml) was collected individually from a subset of five subjects. Each subject provided the sample by allowing saliva to pool at the bottom of the mouth and then expectorating the saliva into a commercially available polypropylene cup. Next, the absorbent swab from saliva collection kit was allowed to saturate with the saliva sample and then precise amounts of saliva (25 µl) expressed by the plunger were collected for subsequent assay. Each harvested saliva sample was treated differently as follows: (a) native saliva only; (b) centrifugation only (1500 g for 15 min); (c) freeze-thawing only (frozen to ~80°C and then thawed at 4°C); and (d) freeze-thaw followed by centrifugation. Subsequently, each saliva sample was divided further into five aliquots (n = 5).

The native saliva obtained by the micropipetting approach was pretreated following the standard processing method for a cortisol ELISA kit and used as a control for establishing concordance. Briefly, a sample was pipetted from the remainder of the passive drool saliva, frozen to ~80°C and then thawed at 4°C in a refrigerator and centrifuged at 1500g for 15 min. A micropipette was then used to remove a fixed aliquot of the saliva (25 µl) for subsequent analysis.

Finally, the sCortisol levels from the individual aliquots (both saliva collection kit and micropipetting) were measured by using a cortisol ELISA kit (Salimetrics LLC, State College, PA) and a plate reader (ARVO MX; Perkin Elmer Life Science, Boston, MA).

**Data analysis**

All analyses were performed with the Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS Inc, Chicago, IL). Linear regression was used to estimate the linear correspondence between the saliva collection kit and the conventional passive drool method. Measurement precision was quantified with the coefficient of variation (CV).

**RESULTS**

Accuracy and precision of the saliva collection kit was established by comparing incremental volumes collected by the kit (3, 10, and 30 µl) from both the cortisol standards and the whole saliva samples. For the cortisol standard, the measured volumes were 2.91 ± 0.19, 9.75 ± 0.43, and 29.64 ± 0.91 µl respectively, with a CV of 6.36% (Fig. 2A). For the whole saliva samples, the measured volumes were 3.85 ± 0.28, 10.79 ± 0.95, and 31.18 ± 1.72 µl, respectively, with a CV of 8.76% (Fig. 2B).

The regression analysis revealed a close correspondence between the sCortisol levels measured from the saliva samples obtained by the collection kit and by the passive drool method. The coefficient of linearity (R² > 0.96) was consistently high for all four pretreatment conditions (0.97, 0.96, 0.98, and 0.97, respectively) and the CVs ranged from 5.01 to 9.56 for the four pretreatment conditions (7.86, 9.56, 6.63, and 5.01, respectively).

**DISCUSSION**

Our results indicate that the new saliva collection kit allows accurate and repeatable collection of fixed amounts of whole saliva and does not interfere with subsequent measurements of salivary cortisol. In our validation testing using both whole saliva samples and control samples,
the volumes dispensed by the kit were very reproducible and closely approximated the graduation marks on the sample tube. Unlike passive drool techniques that can be embarrassing to the subjects, the saliva samples dispensed by the collection kit were easily collected and devoid of any particulates or air bubbles. With the exception of subjects with dry mouth and greatly diminished saliva flow rates, the collection system is capable of saliva volumes adequate for most assays. Furthermore, the non-reactive absorbent swab did not distort quantitative estimates of the salivary analytes, a concern highlighted by Shirtcliff et al. (2001) with regard to conventional cotton absorbent pads. Using salivary cortisol as an example, we verified that immunoassay results from saliva samples collected by our kit corresponded closely to sCortisol levels from samples collected by the conventional passive drool technique, even after various pretreatment steps.

The reproducible collection, seamless recovery of samples, and accurate dispensation of saliva volumes bodes well for the reliability of subsequent assays. A streamlined collection process, lack of any elaborate specimen recovery steps, and the short turnaround time (<3 min) for retrieving the samples should render the kit attractive to test subjects and researchers alike. The immediate availability of accurate amounts of saliva samples is particularly suitable for point-of-care analysis of disease biomarkers through emerging biosensor technologies (Yamaguchi and Shetty, 2012). The simple, inexpensive and user-centric nature of the disposable collection kit should facilitate saliva sample collection in naturalistic settings for large population studies or studies with a repeated measures design.

LITERATURE CITED
Granger DA, Johnson SB, Szanton SL, Ost D, Schumann LL. 2012. Incorporating salivary biomarkers into nursing research: an overview and review of best practices. Biol Res Nurs 14:347–356.
Granger DA, Kivlighan KT, Fortunato C, Harmon AG, Hibel LC, Schwartz EB, Whembolua, GL. 2007. Integration of salivary biomarkers into developmental and behaviorally-oriented research: problems and solutions for collecting specimens. Physiol Behav 92:583–590.
Hellhammer DH, Wust S, Kudielka BM. 2009. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology 34:163–171.
Kaushik A, Vasudev A, Arya SK, Pasha SK, Bhansali S. 2014. Recent advances in cortisol sensing technologies for point-of-care application. Biosens Bioelectron 53:499–512.
Kirschbaum C, Hellhammer DH. 1994. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. Psychoneuroendocrinology 19:313–333.
Koh DSQ, Koh, GCH. 2007. The use of salivary biomarkers in occupational and environmental medicine. Occup Environ Med 64:202–210.
Mandel ID. 1993. Salivary diagnosis: promises, promises. Ann. NY Acad Sci 694:1–10.
Shirtcliff EA, Granger DA, Schwartz E, Curran MJ. 2001. Use of salivary biomarkers in biobehavioral research: cotton-based sample collection methods can interfere with salivary immunoassay results. Psychoneuroendocrinology 26:165–173.
Yamaguchi M, Shetty V. 2012. Salivary sensors in point-of-care testing. In: Distributed diagnosis and home healthcare (D2H2). pp 1–9.
Zhang A, Sun H, Wang X. 2012. Saliva metabolomics opens door to biomarker discovery, disease diagnosis, and treatment. Appl Biochem Biotechnol 168:1718–1727.