Qeios

Peer Review

Review of: "Optimization of Pre-Sampling Conditions for Salivary Genomic DNA Extraction: A Single-Donor Analysis"

Giovana Weckwerth[1]

1. Pharmacology, Universidade de São Paulo, Brazil

The methodology of this article is organized and well described technically, but it presents important methodological limitations. For a pilot study, it is acceptable; however, for publication in a more robust journal or rigorous academic evaluation, there are several flaws.

Main methodological errors and limitations:

1. The study was conducted with only one participant.

I consider this the biggest problem with the study, having been conducted with a “single healthy adult volunteer.” This drastically limits the external validity, the generalizability of the results, and the clinical applicability. Although the authors acknowledge this in the discussion, the problem remains extremely relevant. The results obtained from this study may refer to the salivary characteristics of the individual studied, related to their personal habits, such as their own oral epithelial desquamation.

2. Inadequately randomized experimental design

Throughout the text, the authors describe that: “each pre-sampling condition was tested on separate days.” This is not adequate because variations in the patient's salivary production may occur over the days due to their diet and hydration, as well as the influence of stress and the circadian rhythm on salivary secretion. The authors should perform randomization or temporal control of sample collection.

3. Lack of Circadian Control

The authors describe that they collected samples “1 hour after completion.” However, they do not clarify the time of collection, whether the patient was fasting, what they were eating, or whether their sleep

 doi.org/10.32388/JF1YPD

pattern or brushing schedule was analyzed. Since saliva is strongly influenced by the circadian cycle, these factors can alter the volume, protein and cytokine concentration, quantity, and viscosity of saliva. I consider this a significant limitation.

4. Lack of Standardization

The authors mention using “Listerine® Cool Mint” for gargling, but this solution contains alcohol, essential oils, and antimicrobial substances. Therefore, it is not a neutral variable. Gargling with this substance influences oral cell shedding, cell integrity, oral microbiota, and the amount of salivary proteins. Gargling could be done with water or an antiseptic, presenting a comparison of the differences between two groups, for example.

5. Lack of microbiological control

The total amount of DNA was analyzed, but without distinguishing between human and bacterial DNA. This is a bias. Therefore, a specific human qPCR or the use of a specific human nuclear marker, or even bacterial quantification, should have been performed.

6. Conclusion stronger than the data allow

The conclusions stated: “We recommend water intake and gargling...”, but with all the problematic points addressed previously, it would not be advisable for the authors to make such a conclusion. It would be better to use the term: “preliminary evidence suggests...”

7. Insufficient sample size for robust ANOVA

The three-factor ANOVA test with 3 factors and interactions suggests an n=3, and even then it is extremely fragile. Even with statistical significance, the real power is limited.

8. Absence of statistical power calculation

No sample size calculation or statistical power analysis was performed. This compromises the interpretation of the results.

9. “Purity” assessed only by A260/A280

The authors described the use of A260/A280 for purity assessment, but this index is insufficient to assess the real quality of the DNA. It does not adequately detect bacterial DNA, fragmentation, and specific contaminants. We suggest using: TapeStation; Bioanalyzer; downstream qPCR.

10. Lack of blinding

The authors do not mention whether the analyst was blinded or the conditions were masked. This is an experimental bias.

11. Lack of actual food standardization

The authors claim to control water intake: 500 mL. However, they do not report controlling for food intake, time after meals, or physical activity. These factors can alter saliva.

Despite the limitations, there are good methodological aspects, such as the detailed and reproducible protocol, which was also very well described technically. Furthermore, the project has adequate ethical approval and also used Qubit, which is better than just NanoDrop. The authors also clearly state the limitations of the study in their discussion, and the experimental idea is good.

Declarations

Potential competing interests: No potential competing interests to declare.