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Optimized RNA extraction strategy from whole human saliva

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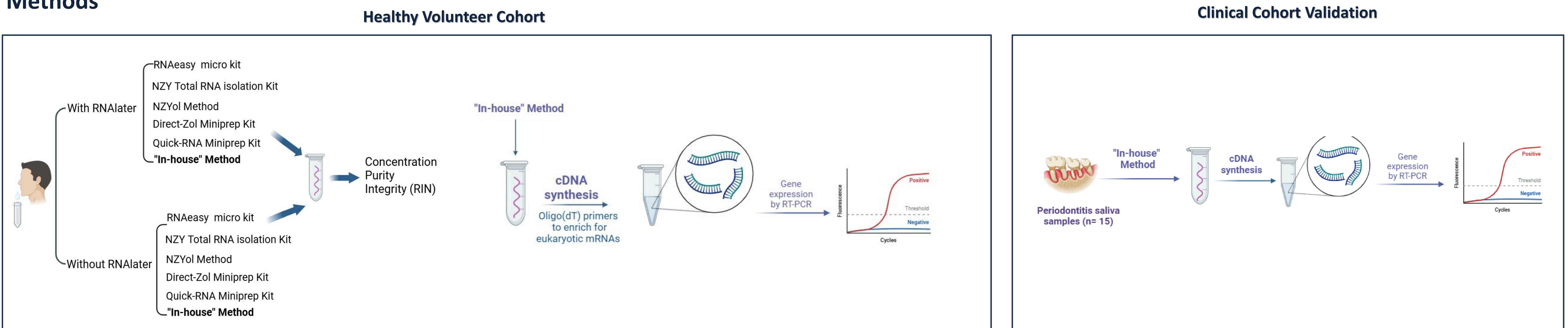
Introduction

Saliva has emerged as a potential source of biomarkers to predict, diagnosis and monitor several diseases particularly within the oral cavity (Melguizo-Rodríguez et al., 2020). Using saliva to detect and quantify biomarkers offers numerous benefits over blood collection because sample is collected by a noninvasive and painless method, allowing the screening of biomarkers even in children. Moreover, saliva can be used for biomarker quantification in the context of clinical diagnosis, prognosis and disease and/or treatment monitoring of oral and systemic diseases (Song et al., 2023; Dongiovanni et al., 2023). However, saliva is a complex biofluid composed by DNA, proteins, RNA, metabolites among others, imposing experimental challenges when isolating nucleic acids, mainly RNA.

This work reports different protocols to extract RNA from human saliva with and without a stabilization solution (RNAlater®, Thermo Fisher Scientific) to evaluate the best RNA extraction method for downstream gene expression studies by real-time PCR (RT-PCR). For this purpose, several parameters as RNA quality, integrity, and yield were assessed.

Methods

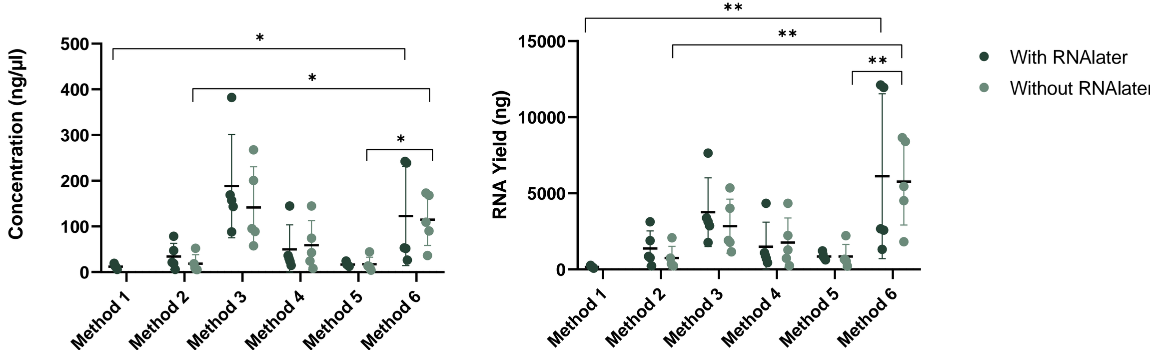


Results

Healthy Volunteer Cohort

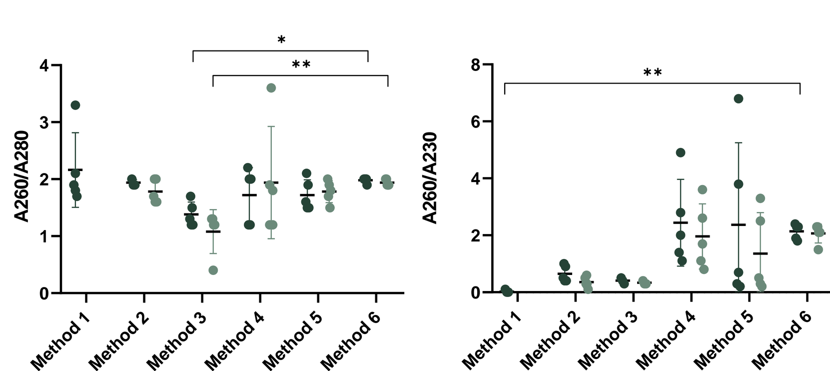
- The "in-house" method revealed the most robust and reproducible results mainly regarding RNA purity ratios compared to the other RNA extraction strategies
- For all tested RNA extraction protocols, no statistically significant differences between samples preserved with or without RNAlater solution were obtained
- RIN (RNA integrity number) from Bioanalyzer is not representative of RNA integrity in saliva samples

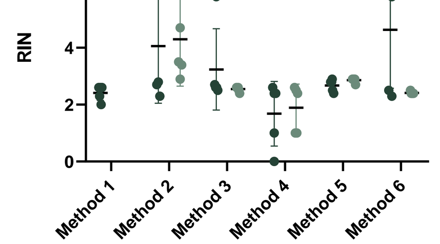
RNA Concentration and Yield



	RNA Extraction Method
Method 1	RNeasy Micro Kit, Qiagen
Method 2	NZY Total RNA Isolation Kit, NZYTech
Method 3	NZYol method, NZYTech
Method 4	Direct-Zol Miniprep Kit, Zymo Research
Method 5	Quick-RNA Miniprep Kit, Zymo Research
Method 6	"In-house" method, alkaline extraction + Quick-RNA Miniprep Kit (Zymo Research)

RNA Purity and Integrity (RIN)





The algorithm used by the bioanalyzer is not suitable for samples containing both human and bacterial RNA so RIN may not be indicative of the integrity of the RNA in saliva samples

Figure 1 – RNA concentration, Yield, Purity (A260/A280 and A260/A230) and Integrity (RIN) of five healthy volunteer saliva samples preserved with or without RNAlater for all tested RNA extraction protocols. *p < 0.05; **p < 0.01. Of note, in method 1 the RNA extraction was only performed with the stabilization solution included in this kit

Healthy Volunteer Cohort - RT-PCR results

- Cq Mean values of five samples showed no significantly differences between saliva preserved with or without RNAlater solution for the three genes analyzed
- Melting curves for each gene confirmed a specific RT-PCR product

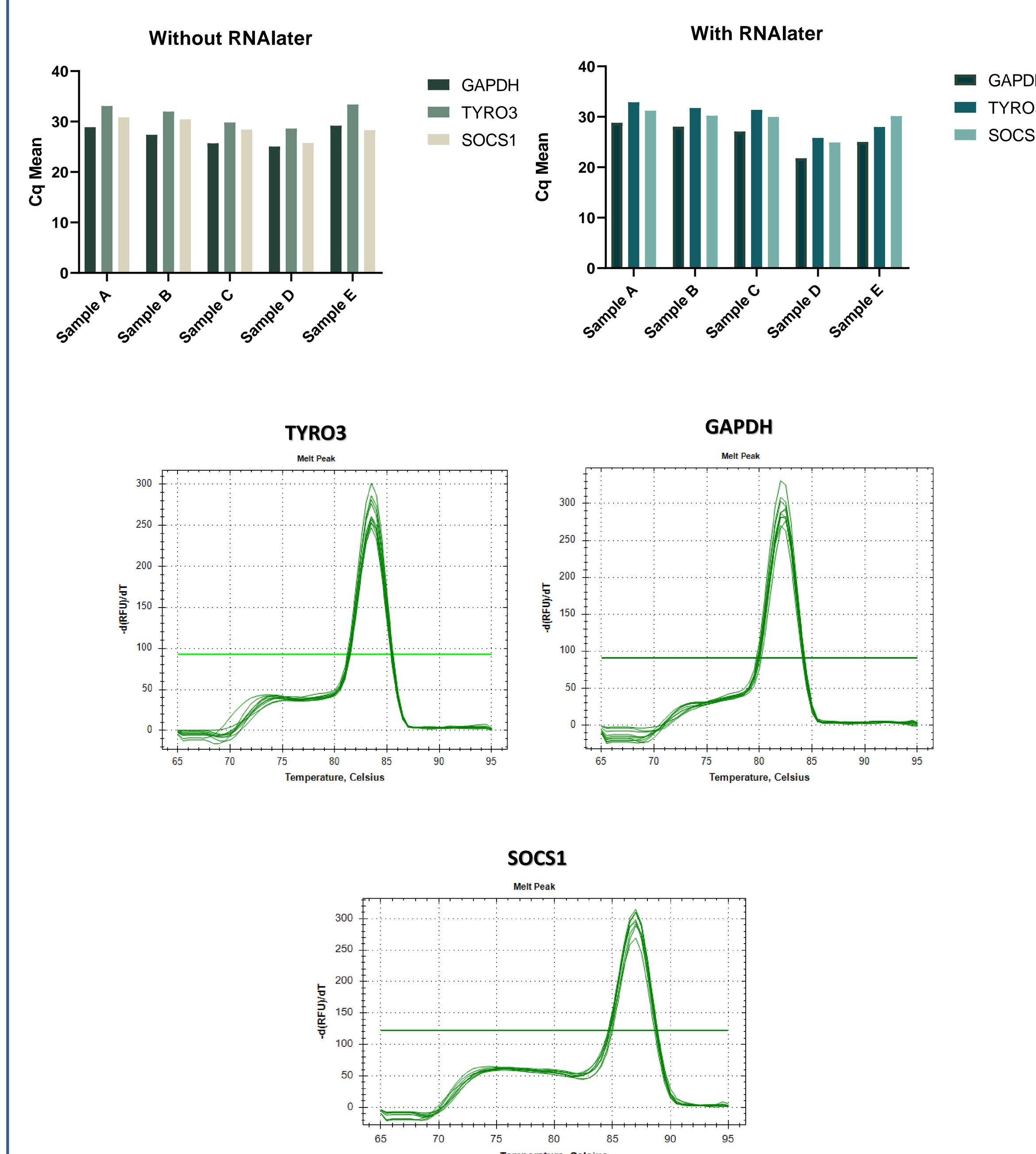


Figure 2 – Cq Mean values for RT-PCR of five RNA samples prepared using the "in-house" method with or without saliva preservation in RNAlater solution. Melting curve of all samples (without RNAlater) to the respective genes

Clinical Cohort Validation

- The "In-house" method produced consistent RNA concentration values and RNA purity ratios for all analyzed periodontitis samples
- RT-PCR results showed reproducible Cq Mean values for each gene among the different periodontitis samples

Table 1 – RNA concentration and purity ratios (A260/A280; A260/A230) (µDrop Plate) of fifteen RNA periodontitis samples prepared using the "In-house" method (without RNAlater solution).

Sample	Concentration (ng/µl)	A260/A280	A260/A230
Sample 1	327	1.9	2.4
Sample 2	198.8	1.9	2.3
Sample 3	579.3	1.9	2.4
Sample 4	107.2	1.9	1.8
Sample 5	645.4	1.9	2.4
Sample 6	335.8	1.9	2.4
Sample 7	556	1.9	2.3
Sample 8	287.6	1.9	2.4
Sample 9	192.5	1.9	2.1
Sample 10	251.7	1.9	2.4
Sample 11	302.5	1.9	2.4
Sample 12	545.3	1.9	2.5
Sample 13	230.6	1.9	2.1
Sample 14	35.6	1.9	1.8
Sample 15	275.4	1.9	2.3

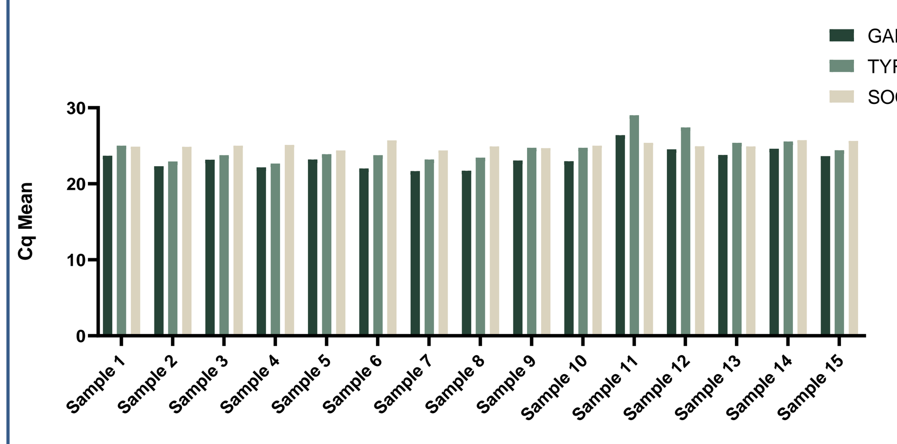


Figure 3 – Cq Mean values for RT-PCR of fifteen RNA samples prepared using the "In-house" method without RNAlater saliva preservation.

Conclusion

The "In-house" method revealed to be the most robust, reproducible, and cost-effective strategy to extract RNA from human saliva. On opposite to other protocols, it does not use toxic chemicals like phenol and chloroform (Gandhi et al., 2020). The purified RNA was successfully used to perform RT-PCR assays for a set of genes, revealing consistent Cq values for all samples analyzed independently whether saliva samples were initially preserved with or without RNAlater solution. Overall, the "In-house" strategy has the potential to quantify salivary genetic biomarkers in a clinical setting contributing towards the development of precision medicine for oral and systemic diseases.

References

MELGUIZO-RODRÍGUEZ, L. et al. Salivary Biomarkers and Their Application in the Diagnosis and Monitoring of the Most Common Oral Pathologies. International Journal of Molecular Sciences, 21(14): 5173, 2020.

SONG, M. et al. Promising applications of human-derived saliva biomarker testing in clinical diagnostics. International Journal of Oral Science, v. 15, n. 1, p. 2, 2023.

Dongiovanni, P. et al. Salivary biomarkers: novel noninvasive tools to diagnose chronic inflammation. International Journal of Oral Science, v. 15, n. 1, p. 27, 2023.

Gandhi, V. et al. High-Quality and High-Yield RNA Extraction Method From Whole Human Saliva. Biomarker Insights, 15, 1–9, 2020.

Ethical declaration

The ethical aspects of the present study were reviewed and approved by the Ethics Committee for Health at Universidade Católica Portuguesa approved under the project OralPreciseMed (nº157).

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