Faculty of Medicine and Dentistry
Palacký University Olomouc

IMTM

Comparative Analysis of Protein Extraction from Saliva and GARGTEST

Ema Heffnerová, Jana Václavková, Marián Hajdúch, Petr Džubák

1 Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Olomouc Czech Republic

Introduction

Saliva is a biological fluid composed of diverse range of molecules, such as enzymes, proteins, electrolytes, mucopolysaccharides, antimicrobial peptides and nucleic acids. In addition to its vital physiological functions in oral defense, lubrication, and digestion, saliva can be used as diagnostic medium. Its easy, safe and non-invasive collection, combined with the ability to reflect local and systemic physiological states, makes it an attractive alternative to blood or tissue samples. Salivary analysis has been explored in the diagnosis of cancer, neurodegenerative disorders, autoimmune diseases, human immunodefi ciency virus (HIV), cardiac disease and oral health monitoring.

However, saliva is a highly dynamic and variable fluid. Factors such as stimulation, collection technique, circadian rhythm, and donor hydration can significantly influence its volume and molecular composition. These variations may affect protein yield and quality, thereby impacting downstream proteomic and biomarker analyses.

We compared proteins extracted from samples obtained GARGTest, a gargle-based test containing a stabilizing powder, and samples obtained by non-stimulated saliva collection.

Methods

To compare the proteome obtained by 2 sampling methods we collected GARGTEST and non-stimulated saliva samples from 6 healthy volunteers aged 25 to 40 years. Participants were asked to rinse their mouths with water and expectorate saliva into a 50mL Falcon tubes. For the GARGTEST, participants gargled tap water for at least 30 seconds and then spat the gargled contents into a container containing stabilizing medium. Saliva and GARG samples were stored at −20°C .

Proteins were extracted from the samples using the TRI-reagent method. Protein concentration was measured by Pierce™ BCA Protein Assay Kit. The extracted proteins were subsequently reduced, alkylated and digested in solution using trypsin. Finally, the samples were desalted using OMIX tips.

Tryptic peptides were analysed using a Dionex Ultimate 3,000 nano-LC system connected to an Orbitrap Fusion Tribrid Mass Spectrometer (Figure 1).

Since protein yields from GARGTEST after extraction using the Tri-reagent method were low, we also tried extraction using FASP **(**Filter-Aided Sample Preparation).

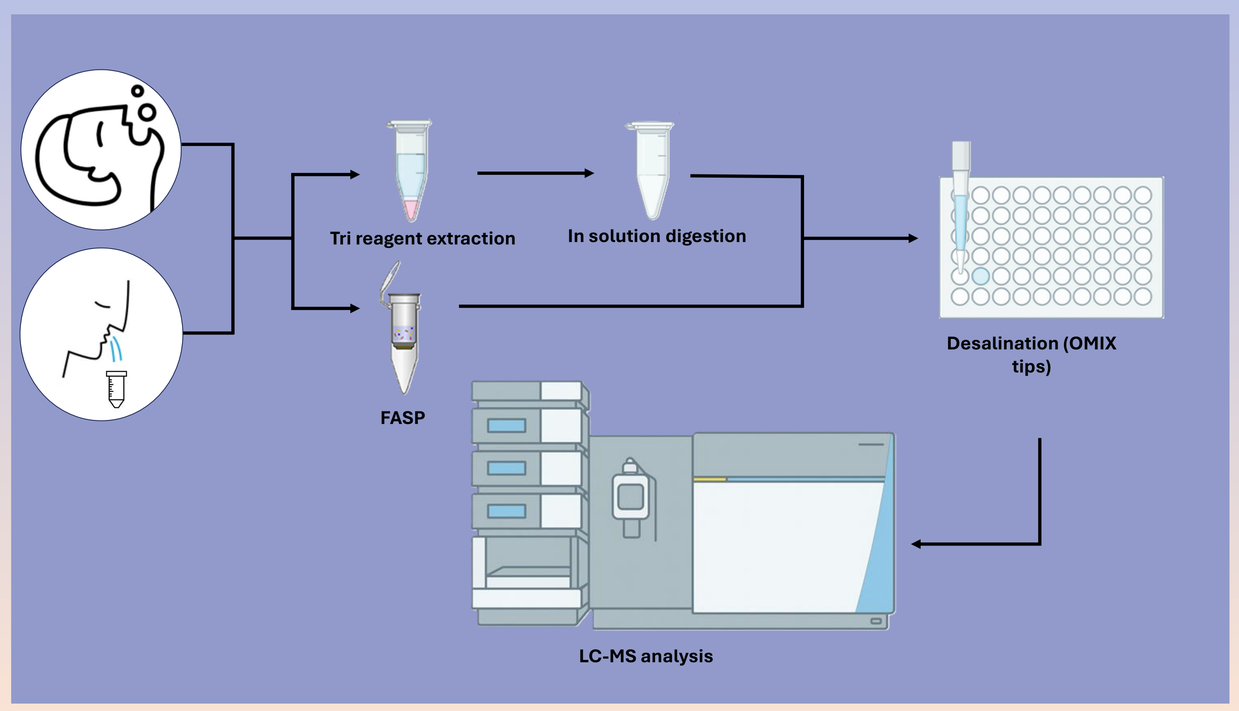


Figure 1|Workflow of protein extraction and analysis

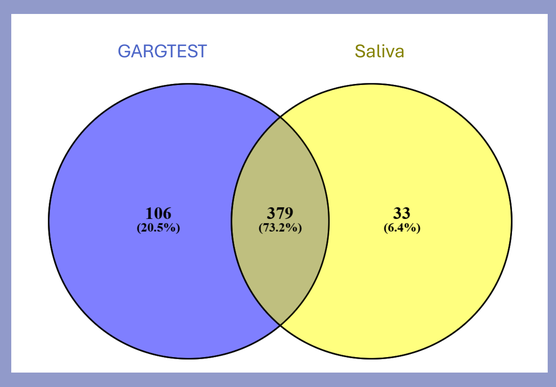
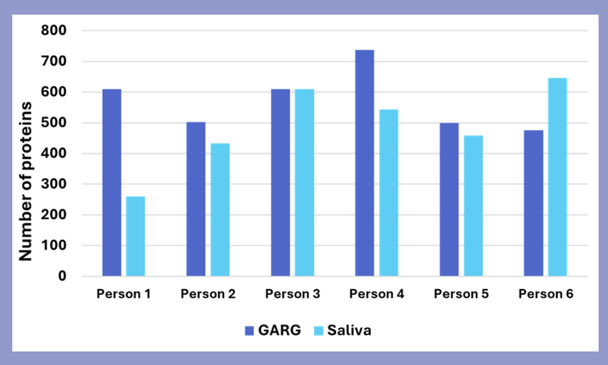


Figure 2 | Venn diagram showing the number of proteins identified in gargle and saliva samples

Figure 3 |Number of identified protein groups in gargle and saliva samples across six individuals.



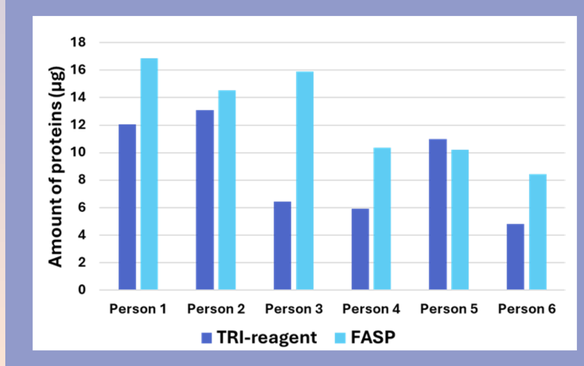


Figure 4 |Protein yield obtained using TRI-reagent and FASP extraction across six individuals.

Conclusions

The comparison of gargle and saliva proteomes shows a high degree of overlap, with the majority of proteins (**73.2%**) detected in both sample types. However, each sample type also contains a distinct subset of proteins, with gargle samples yielding a larger number of unique identifications. These findings indicate that while both matrices are suitable for proteomic analysis, the gargle method may provide broader proteome coverage.

Additionally, the FASP method used for protein extraction delivered more stable results. FASP also proved to be a faster and more time-efficient workflow, making it advantageous for high-throughput proteomic analysis.

Contact: emaheffnerova01@upol.cz

This work was supported by the Technology agency of the Czech Republic project PERMED: T2BA (TN02000109). We also acknowledge the contributions from infrastructural project EATRIS-CZ (LM2023053) and IGA_LF_2025_021. We are also grateful for the support from project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102)

Funded by the European Union NextGenerationEU
NÚVR NÁRODNÍ ÚSTAV PRO VÝZKUM RAKOVINY
PerMed T2BA
eatris-cz European infrastructure for translational medicine Czech Republic
MŠMT MINISTRY OF EDUCATION, YOUTH AND SPORTS