



# Spit Tests: Biomarkers in the Salivary Proteome



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Saliva is a readily accessible body fluid whose composition is likely to alter in diseased states. This project aims to identify salivary and sputum biomarkers for a range of diseases including gingivitis, cystic fibrosis and asthma using label-free mass spectrometry.

## 1. Salivary biomarkers



Saliva is easy to ship and store and can be obtained at low cost and in sufficient quantities for analysis.<sup>1</sup>

Gingivitis and periodontitis are gum diseases with symptoms including bleeding, swollen and receding gums. There is a need to identify reliable markers for disease activity and to assess the efficiency of treatments.<sup>2</sup>

Pooled whole saliva samples from gingivitis and periodontitis patients are compared with control samples. These samples are obtained from P&G (Procter & Gamble) and EDI (Eastman Dental Institute).

## 2. Saliva bacterial and human proteome

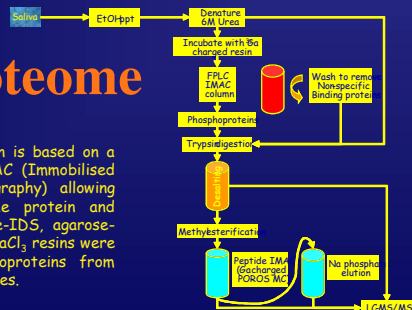
It is difficult to search for salivary biomarkers as there are a large number of proteins in saliva (ca. 1500)<sup>3</sup> and it is not possible to analyse all 1500 in 1 LCMS (liquid chromatography-mass spectrometry) run. We are therefore focussing on a small subset of proteins, phosphoproteins, to simplify the dataset. The degree of phosphorylation is thought to change with the onset of various diseases.

As well as profiling the human portion of the salivary proteome, the bacterial proteins in oral fluids will also be investigated. For diseases which are thought to be predominantly caused by changes in bacterial populations, such as gingivitis and periodontitis, there is the potential to identify biomarkers in the changing proportions of bacterial proteins.<sup>4</sup>

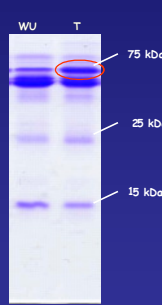


## 3. Salivary phosphoproteome

Phosphoprotein extraction is based on a protein and peptide IMAC (Immobilised meal affinity chromatography) allowing enrichment at both the protein and peptide level.<sup>5</sup> Sepharose-IDS, agarose-nitrotriacetic acid and GaCl<sub>3</sub> resins were used to isolate phosphoproteins from urea-soluble protein samples.



## 4. Biomarker searching strategy

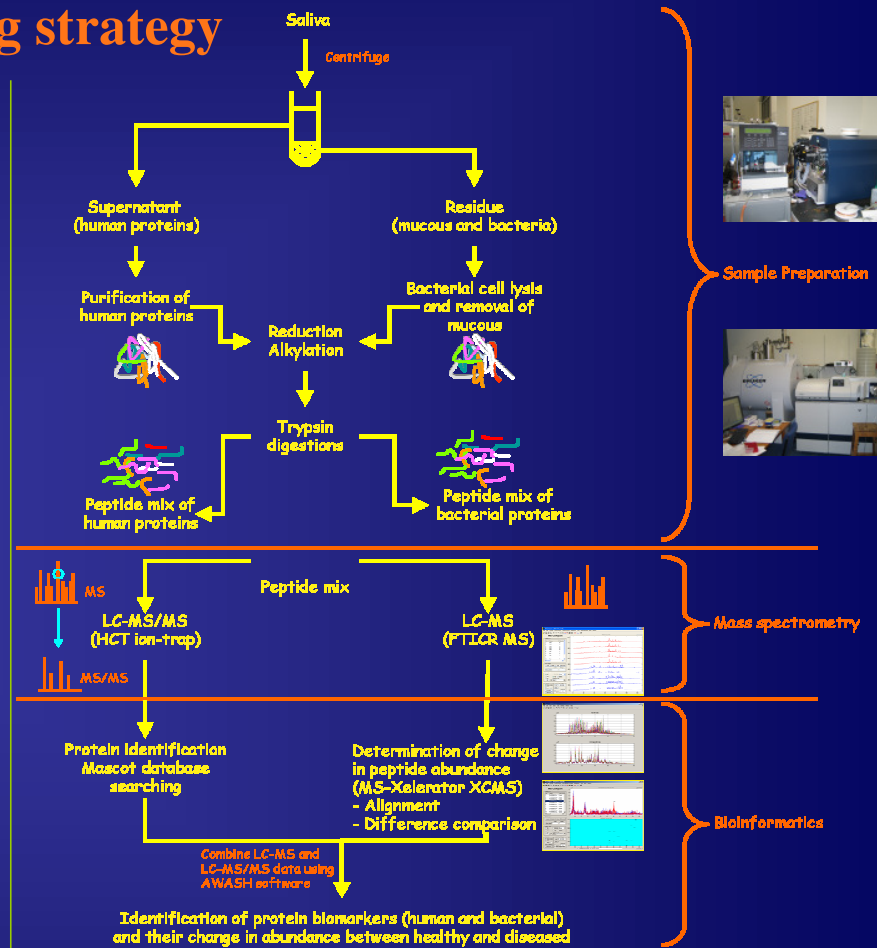
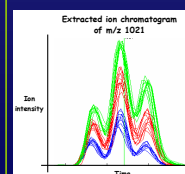
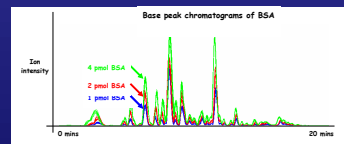
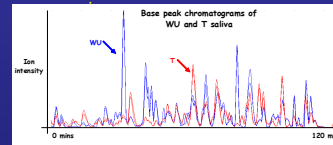


Label-free relative quantitation using the LC-MS (liquid chromatography mass spectrometry) is being carried out to generate a list of putative biomarkers. These potential biomarkers are then identified using LC-MS/MS (liquid chromatography tandem mass spectrometry) on the same sample.

For method development two saliva samples with significant differences, as identified by SDS-PAGE, are being used. The two samples are saliva taken at 'wake-up' (WU) and 'after toothbrushing' (T) from the same individual. These two samples (both whole saliva and digested) have been run on an ion-trap (HCT, Bruker) and a 12T FTICR (fourier transform ion cyclotron resonance) mass spectrometer in both LC-MS and LC-MS/MS mode and the data has been analysed using 3 different softwares:

- XCMS (Scripps Institute)<sup>6</sup>
- Progenesis (Non-linear dynamics)<sup>7</sup>
- MS-Xelerator (MsMetrix)<sup>8</sup>

In-house software has been written to combine the LC-MS quantitation data with the identification data from the LC-MS/MS.



Sample Preparation



Mass spectrometry

Bioinformatics

- Saliva spiked with 2 different concentrations of BSA - expected differences observed in presence of saliva.
- Significant changes observed between wake-up (WU) and after-toothbrushing (T) saliva.
- For peaks with sensitivity >1% 264 significant differences between WU and T (whole) saliva observed.
- Linking of quantitative LCMS and LCMSMS identification data still underway.

## References and Acknowledgements

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