# SalivaTec: using xMAP® Multiplex Technology for saliva research

N. ROSA[1*], M. CORREIA[1], M. BARROS[1]

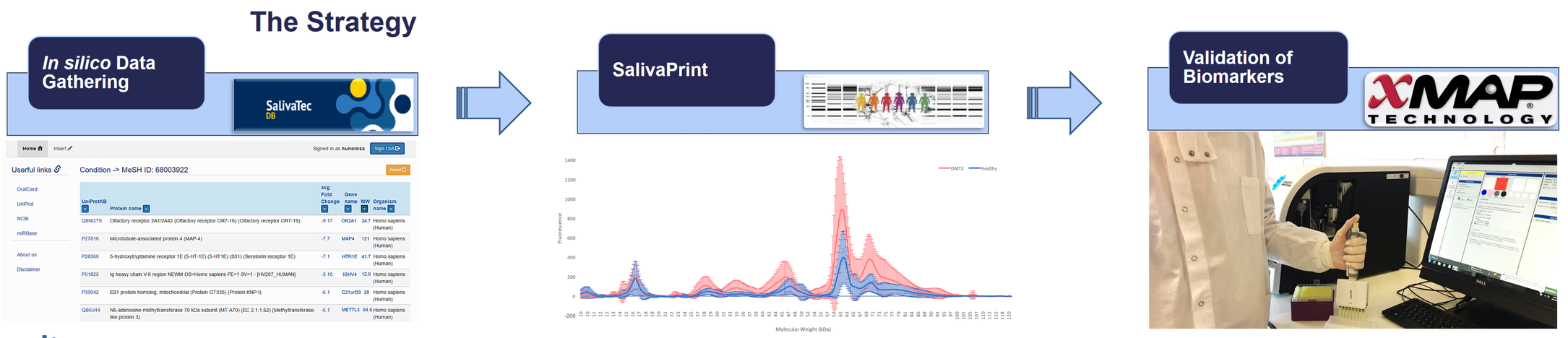
[1]Universidade Católica Portuguesa, Center for Interdisciplinary Research in Health, Institute of Health Sciences, Viseu, Portugal;

## Background and Aim

- SalivaTec is an European laboratory using saliva in health research (salivaTec.viseu.ucp.pt). The main goal of SalivaTec is the clarification of molecular mechanisms of disease and the validation of biomarker panels proposed by *in silico* studies using bioinformatics tools developed in the laboratory. We demonstrate how SalivaTec uses xMAP ® Multiplex technology to quantify different molecules in saliva. These molecules are selected by an *in silico* analysis of the data deposited in our databases and quantified in samples chosen through the SalivaPrint algorithm, which identifies the molecules varying the most between groups of individuals. xMAP technology allows for the detection of salivary markers with high sensitivity and reliability although some challenges remain in the application of the commercial kits to saliva.

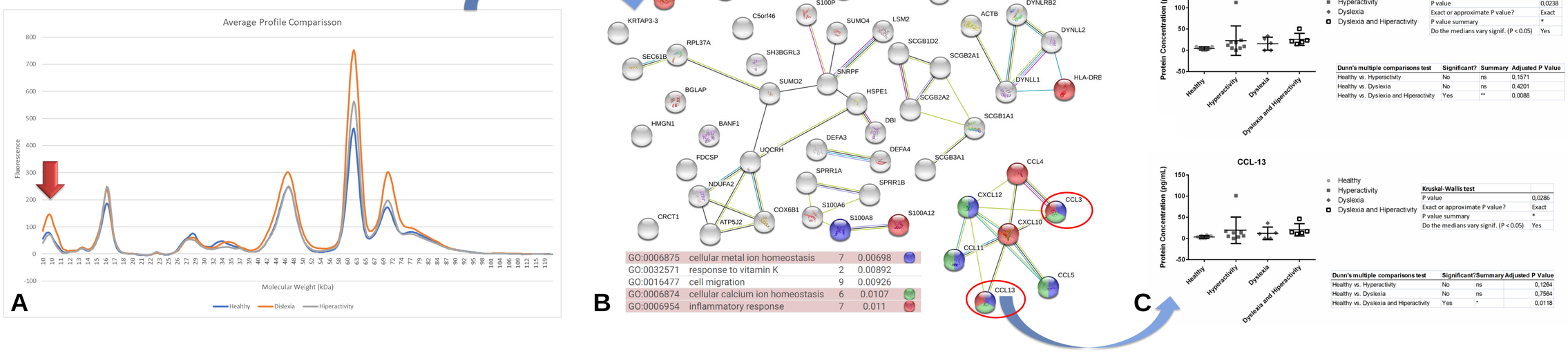
## Methods

- The strategy followed starts with an *in silico* analysis of the data in SalivaTecDB[1] which identifies the potential molecules representing molecular mechanisms involved in the conditions under study. Additionally, a protein profile is obtained for each individual by capillary electrophoresis, generating a "SalivaPrint"[2] of each subject which enables the selection of representatives from each group. These molecules are quantified in saliva using different **xMAP® Multiplex** immunoassay kits from Biorad® and Millipore®.

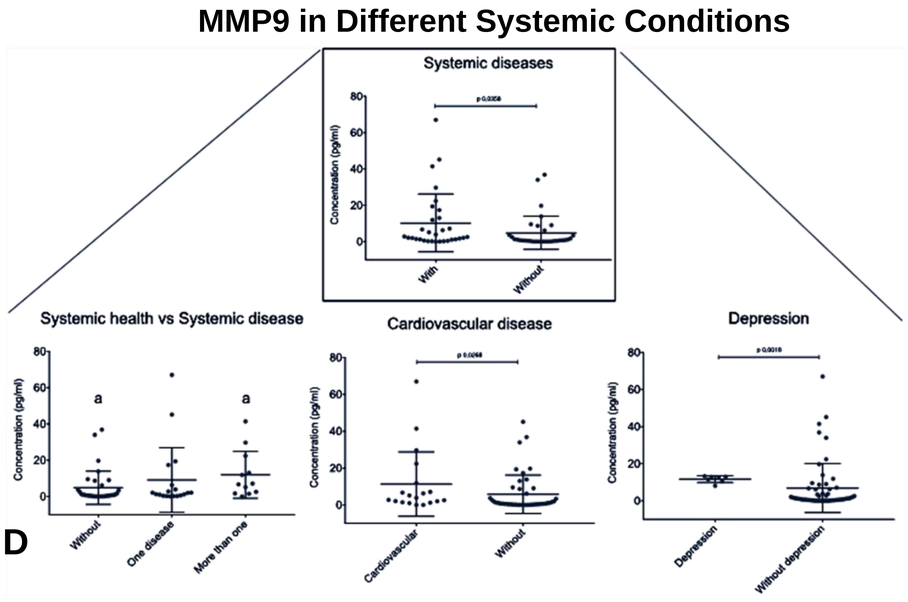


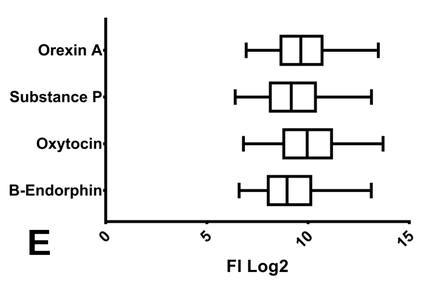

## Results

- Results for the quantification of cytokines in using Bio-Plex Pro™ Human Th17 Cytokine Panel 15-Plex (**F**), Bio-Plex Pro™ Human Chemokine MIP-1α/CCL3 (**C**) and Bio-Plex Pro™ Human Chemokine MCP-4/CCL13 (**C**) all from Biorad® and Milliplex HSTCMAG-28SK-03 Hu (**F**). High Sens. T Cell Mag from Millipore® are presented. We also discuss results for the quantification of Oxytocin, Orexin A, β-Endorphin and Substance P using Milliplex HNPMAG-35K kit from Millipore® (**E**)**,** as well as the quantification of MMP9 using the Biorad kit Bio-Plex Pro™ Human (**D**). All quantifications were performed in saliva samples following the manufacturer's instructions for serum samples, since there were no specific recommendations for saliva.

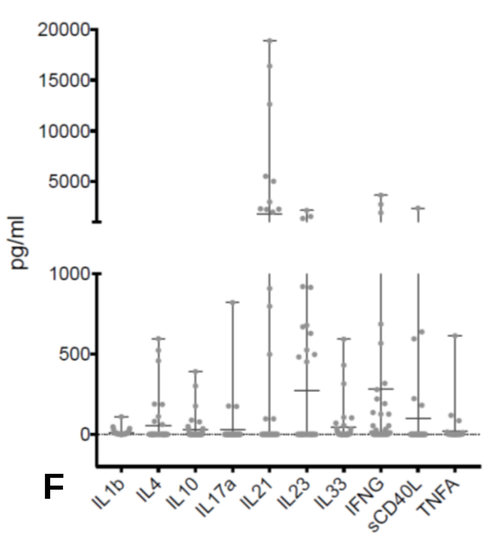



Results from the application of the SalivaTec strategy to the study of Dislexia and Hyperactivity[3]. **A)** SalivaPrint[2]: Choice of MW range distinguishining dofferent study conditions; **B)** Functional analysis (STRING[4]) for the identification of potential biomarkers to be quantified by Multiplex; **C)** Multiplex quantification of the potential biomarkers proposed.




**Quantification of different molecules in saliva samples through xMAP® Multiplex Technology (D, E, F)**




| Molecule | IL1b | | | | | | | | | | | IL4 | | | | IL6 | | | | | | | | | | IL10 | | | | | IL17 | | IFNg | | TNF-a | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | Teles, 2009 | Chang, 2011 | Fancourt, 2016 | Twal, 2016 | Gursoy, 2014 | Jorgensen, 2016 | Filaire, 2011 | Shields, 2016 | Soto-Méndez, 2015 | Browne, 2013 | Fancourt, 2016 | Filaire, 2011 | Thomas, 2009 | Suh, 2009 | Soto-Méndez, 2015 | Teles, 2009 | Chang, 2011 | Fancourt, 2016 | Gursoy, 2014 | Jorgensen, 2016 | Filaire, 2011 | Suh, 2009 | Soto-Méndez, 2015 | Browne, 2013 | Fancourt, 2016 | Teles, 2009 | Gursoy, 2014 | Twal, 2016 | Thomas, 2009 | Filaire, 2011 | Twal, 2016 | Suh, 2009 | Soto-Méndez, 2015 | Filaire, 2011 | Jorgensen, 2016 | Fancourt, 2016 | Twal, 2016 | Filaire, 2011 | Thomas, 2009 | Suh, 2009 | Soto-Méndez, 2015 | Browne, 2013 | Fancourt, 2016 |
| [IL] in pg/mL (mean, median) | 1.7 | 2.8 | 8.9 | 259.7; 84.9 | 26.3 | 32 | 625 | 4.9 | 61.3; 38.1 | 24.5 | 18.3 | 110 | 54.3 | 1.6 | 26; 5 | 1.4 | 1.3 | 10.1 | 9.5 | 10 | 40 | 1.5 | 14.2 ;4.1 | 27.6 | 15.9 | 11 | 10.3 | 9.5; 1.6 | 50.1 | 350 | 36.1; 0 | 0.8 | 21.5; 0.8 | 125 | 3 | 2.3 | 80; 11.2 | 130 | 55.1 | 1.2 | 20.6; 9 | 17.9 | 2.4 |
| Min. (pg/mL) | 0.3 | | | 1.4 | 15.2 | 0 | | | 0.5 | | | | | | 0.4 | 0.8 | | | | 0 | | | 0.1 | | | 1.2 | | 0 | | | 0 | | 0.1 | | 0 | | 0 | | | | 0.1 | | |
| Max. (pg/mL) | 5.7 | | | 1184.2 | 126.1 | 31623 | | | 421.6 | | | | | | 1854 | 1.4 | | | | 1000000 | | | 621.6 | | | 108.8 | | 1.6 | | | 433.3 | | 2425 | | 316 | | 594.1 | | | | 411.1 | | |
| Donor Age | 4.5 | 18-22 | 26 | 27 | 18-32 | 18-45 | 18-65 | 24-39 | 53-84 | 65.1 | 71 | 18-65 | 41.8 | 52 | 53-84 | 4.5 | 18-22 | 26 | 18-32 | 18-45 | 18-65 | 52 | 53-84 | 65.1 | 71 | 4.5 | 18-32 | 27 | 41.8 | 18-65 | 27 | 52 | 53-84 | 18-65 | 18-45 | 26 | 27 | 18-65 | 41.8 | 52 | 53-84 | 65.1 | 71 |
| Donor Sex | Both | Females | Both | Both | Both | Both | Both | Females | Females | Females | Both | Both | Both | Both | Females | Both | Females | Both | Both | Both | Both | Both | Females | Females | Both | Both | Both | Both | Both | Both | Both | Females | Both | Both | Both | Both | Both | Both | Both | Females | Females | Both |

Literature reported results for different cytokines and sCD40L in healthy individuals determined by Multiplex Technology. **There is a wide variability in the values presented.**

## Conclusions

- **xMAP ® Multiplex** technology, enables the detection and quantification of salivary molecular markers in reduced sample volumes, empowering the use of saliva as a noninvasive biological informative fluid. However, the application of the commercially available kits to saliva is not yet well standardized since most kits are optimized for serum or plasma. Furthermore, the comparison of the results to those reported in the literature is hampered by the fact that different descriptive statistics are used by most authors. Additionally, values of the same molecules seem to vary widely between studies, which raises the question of reproducibility between labs.

## References

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CATÓLICA CIIS · CENTRO DE INVESTIGAÇÃO INTERDISCIPLINAR EM SAÚDE LISBOA · PORTO · VISEU
salivatec@viseu.ucp.pt
http://salivatec.viseu.ucp.pt

SALIVATEC
LABORATORY FOR MULTIDISCIPLINARY RESEARCH IN SALIVA