JOANNEUM RESEARCH HEALTH

Metabolomics in research and diagnostics: The technology is ready - now it's time to standardize

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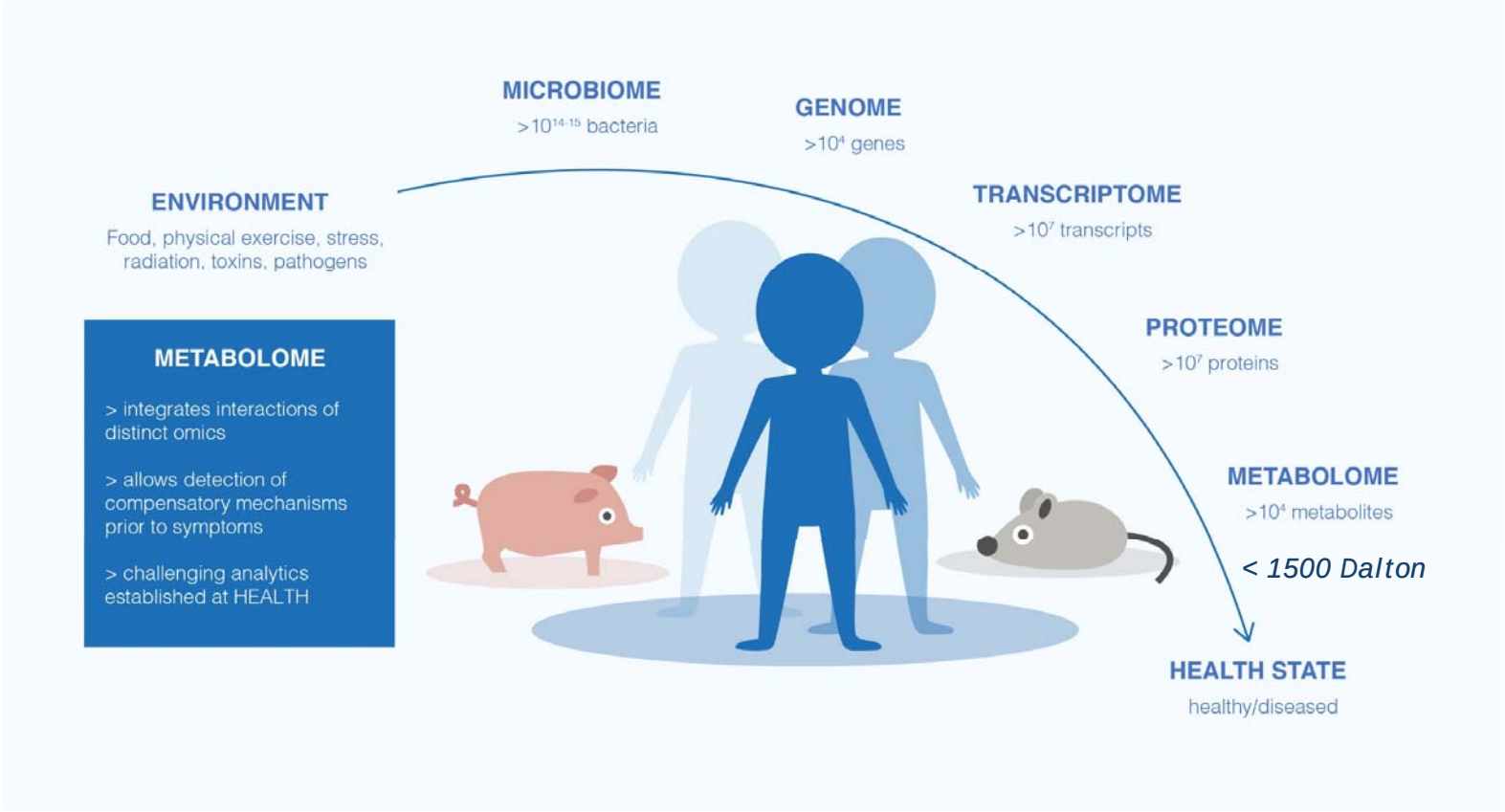
HEALTH - Institute for Biomedicine and Health Sciences – bridges the gap between basic medical research and industrial application.

- We develop, optimize and validate analytical methods for preclinical/clinical studies and pharmaceutical products.
- We combine scientific expertise with service orientation and high quality standards (GLP/GCP).
- We offer analytical solutions for pharmaceutical product development.

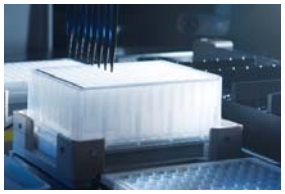
Metabolomics

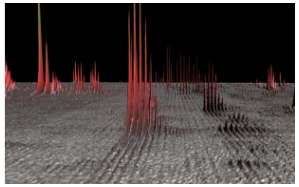


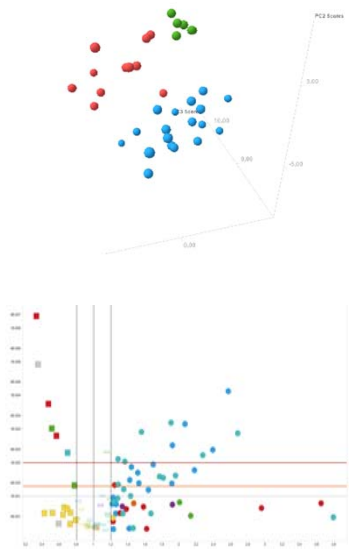
Credit: JOANNEUM RESEARCH

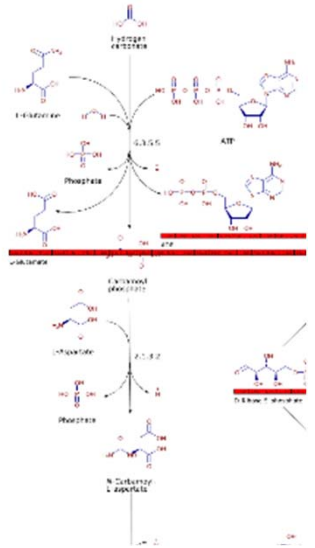
Metabolomics Workflow @ JOANNEUM RESEARCH

Sample Generation	Sample Preparation	Analysis	Data Processing & Statistics	Biological Interpretation
Tissue, tissue biopsies	Extraction of low molecular weight compounds	High Resolution Mass Spectrometry		
Biological liquids (blood, urine, interstitial fluid)	Preparation of QC sample			
Cell culture & supernatant				





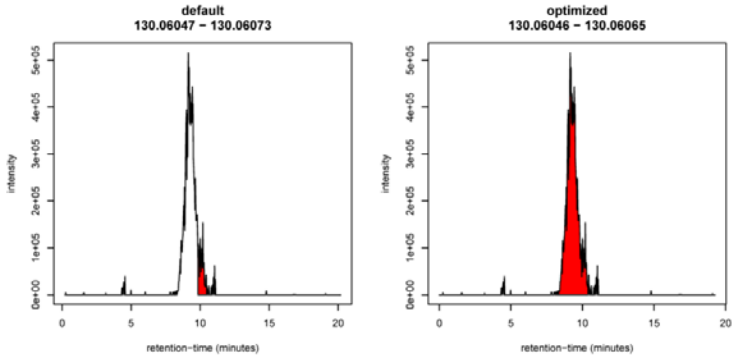




Credit: : https://projects.iq.harvard.edu/files/pfister/files/vis2005_0.pdf

Isotopolouge Parameter Optimization (IPO) developed @JOANNEUM RESEARCH

- JR developed a worldwide used optimization tool for untargeted data processing (XCMS): IPO
  - Open Source; available @ Bioconductor
  - Currently about 200 citations in peer review articles
- Principle: Maximizing of natural isotopic peaks in metabolomics high resolution mass spectrometry data sets



- IPO helped to increase data quality in untargeted metabolomics worldwide
- One step of standardization

Libiseller, G., Dvorzak, M., Kleb, U., Gander, E., Eisenberg, T., Madeo, F., Neumann, S., Trausinger, G., Sinner, F., Pieber, T., & Magnes, C. (2015). IPO: a tool for automated optimization of XCMS parameters. BMC Bioinformatics, 16(1), 118. https://doi.org/10.1186/s12859-015-0562-8
http://bioconductor.org/packages/release/bioc/html/IPO.html

Examples Metabolomics Biomarker

	Metabolites
Parkinson´s Disease	Long-chain acylcarnitine. kynurenic acid, quinolinic acid, ratio of kynurenic acid/kynurenine, ratio of quinolinic acid/kynurenic acid
Alzheimer´s disease	Phophocholines , aycylcarnithines, asparagin. ADMA, Asn, Arg, histamine, citrulline, nitrotyrosine
Diabetic retinopathy	Alterations in glucose and purine metabolism, fumarat, uridine, acetic acid, cytidine, glutamine, glutamate; activation of alanine, glutamate metabolic pathways
Cardiovascular disease	N6, N6, N6-trimethyl-L-lysine, linoleate metabolism, acylcarnithines, sphingomyeline, 3-hydroxybutyrates
Pulmonary Hypertension	Free fatty acids (Patent JR/CBMED/MUG/LBI, WO2017153472A1)
Cancer	Energy metabolism, metabolic reprogramming, Warburg effect

Aderemi, A. V., et al (2021). Metabolites (Vol. 11, Issue 7, p. 418). https://doi.org/10.3390/metabo11070418
García-Cañaveras, J. C. Et al (2021). Cancers, 13(13). https://doi.org/10.3390/CANCERS13133230
Wilkins, J. M., et al (2018). 8(JAN), 719. https://doi.org/10.3389/FNEUR.2017.00719/BIBTEX

Metabolomics Technologies

	Nuclear magnetic resonance (NMR)	Mass spectrometry (MS)
Sensitivity	Low (LOD ~5 µM)	High (LOD ~ 0.5 nM)
Reproducibility	Very high	Average
Number of detectable metabolites	30-100	300-1000+ (depending on whether GC-MS or LC-MS is used)
Targeted analysis	Not optimal for targeted analysis	Better for targeted analysis than NMR
Sample preparation	Minimal sample preparation required	More complex sample preparation required
Sample destruction	No	Yes
Tissue extraction	Not required – tissues can be analysed directly	Requires tissue extraction
Sample analysis time	Fast – the entire sample can be analysed in one measurement	Longer than NMR – requires different chromatography techniques depending on the metabolites analysed
Instrument Cost	More expensive and occupies more space than MS Low availabilty	Cheaper and occupies less space than NMR High availability
Sample Cost	Low cost per sample	High cost per sample

Targeted / Untargeted Metabolomics

Metabolomics

Untargeted / Discovery

- Hypothesis generation
- Best metabolome coverage
- Qualitative identification
- Relative quantification

Targeted / Validation - Application

- Absolute quantification
- Limited number of metabolites
- Identification known
- Hypothesis driven

Comprehensive Targeted / Discovery - Validation - Application

- Hypothesis generation / Hypothesis driven
- Large number of known metabolites (>500)
- Absolute quantification
- Focused to limited sample species (plasma, urine)

Standardization Initiatives

Currently focused to untargeted metabolomics and basic resarch to improve comparability of study results

- **Metabolomics society: Metabolomics Standards Inititative (MSI)**
- **NIH: Metabolomics Quality Assurance & Quality Control Consortium (mQACC)**
- **European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC: MEtabolomics standaRds Initiative in Toxicology (MERIT)**

Gap: Standardization initiatives for targeted metabolomics methods. They are already closer to clinical application

Metabolomics Standardization Needs

Biomarker Validation studies	Specificity and sensitivity of biomarker panels need to be investigated in validation studies: Control groups including healthy individuals, but also diseases and related conditions Diversity of the population must be reflected.
Pre-analytics and sample preparation	Metabolites are sensitive to sampling, sample storage conditions and extraction procedures. These must be standardized (SOPs) to allow comparability among studies.
Analytics	Comparability only possible via absolute quantification. Monitoring of lab-to-lab, instrument-to-instrument and longterm comparability
Reporting	Outliertest, standardized statistical analysis, standardized data formats
For clinical applications	Definition of biomarker signatures and associated causality to disease Reference methods, reference laboratories Available traceable and commutable calibrators and standards Proficiency tests Certified reference materials

DA, D., & T, K. (2016). Progress in Metabolomics Standardisation and its Significance in Future Clinical Laboratory Medicine. *EJIFCC*, *27*(4), 331–343
Viant, M. R.et al. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-10900-y

Wish list to EURAMET

Standardization program for targeted metabolomics to facilitate

- Collaboration between metabolomics laboratories, targeted metabolomics kit developers, reference material providers and study sites
- Round robin tests
- Certificated reference materials (comparable to NIH SRM1950)
- External quality assurance programs