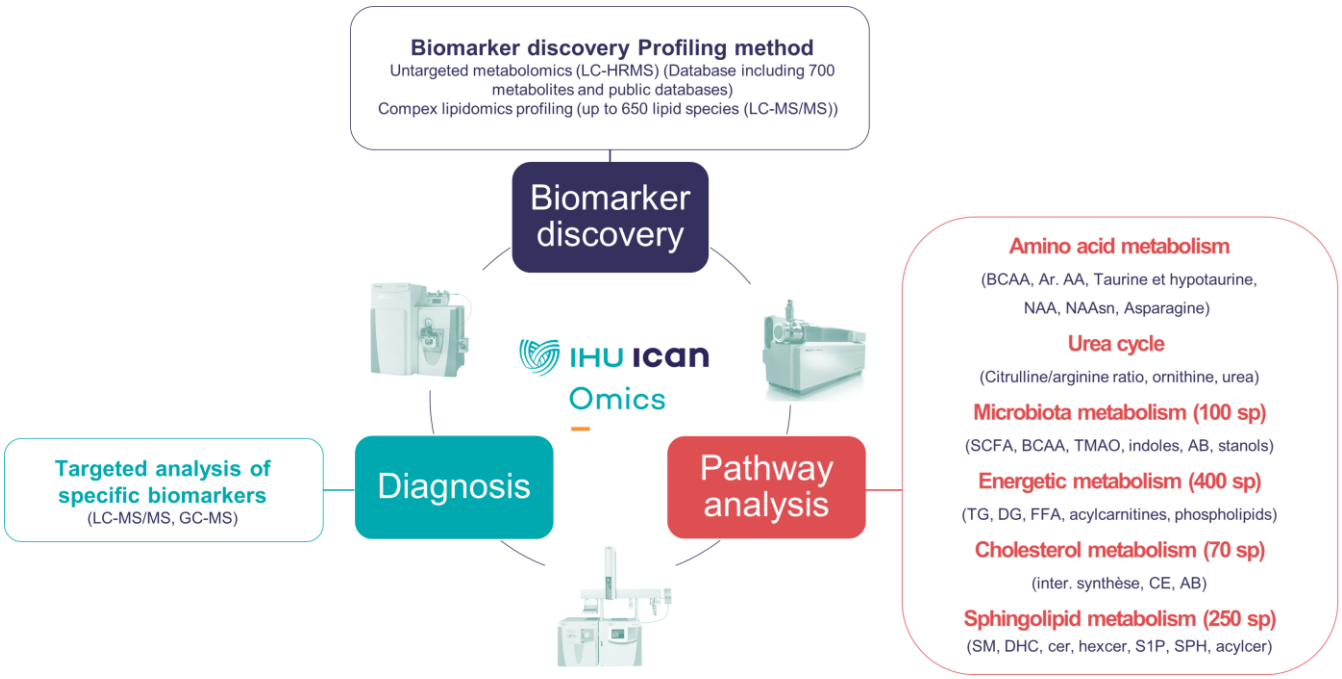
Several approaches were proposed by ICAN Omics metabolomics:

- **Untargeted appraoch for biomarker discovery**
- **Profiling methods for pathways analysis**
- **Targeted approach for candidate marker validation**



Untargeted approach

Aim: Propose a snapshot of the whole metabolome

List of features annotated based on a local and a public database

Local database includes over 650 metabolites + Public database includes over 10.000 chemical entities from KEGG and HmDB databases

Strategy of annotation and identification steps were based on standards proposed by L.W. Sumner et al. 2007 and 2014.

Sample requirements = 100-150 µl Biofluids (plasma/serum, urine), 100mg Faeces, 1.000.000 cells, 50mg of tissue (Liver, brain, heart…)

Profiling approach

Aim: Multiplex analyses of the metabolites usually linked to microbiota and cardiometabolic diseases

Screening of 100-140 metabolites derived from aminoacids metabolism, tryptophane-kynurenine pathways, methylamine metabolism, phenolic compounds, B-vitamins metabolism, short-chain acylcarnitines, organic acids and indoles

Targeted Approach

Aim: Analyses of published markers linked to microbiota activity and cardiometabolic diseases

3 available methods:

- 27 metabolites derived from aminoacids metabolism
- 19 metabolites from the tryptophane-kynurenine pathways
- 6 metabolites derived from dietary choline metabolism
- 6 SCFA metabolites*

Sample requirements per method = 50µl Biofluids (Plasma/Serum/Urine)*, 50mg of faeces, 500.000 cells

Validation only for faeces, caecal content

Tryptophan-kynurenine pathways (19)

- 2-aminoadipic acid
- 3-Hydroxyanthranilic acid
- 3-Hydroxykynurenine
- Anthranilic acid
- Kynurenic acid
- Kynurenine
- Picolinic acid
- Quinaldic
- Quinolinic acid
- Tryptophan
- Xanthurenic acid
- Serotonin
- Melatonin
- Nicotinamide
- Hydroxy-tryptophan
- Nicotinate
- Indole-3-acetate
- Indole-3-lactate
- Indole-propionate

Aminoacids metabolism (27)

- Alanine
- Arginine
- Aspartic acid
- Glutamic acid
- Glycine
- Lysine
- Leucine
- Methionine
- Phenylalanine
- Proline
- Serine
- Threonine
- Tyrosine
- Valine
- Cystine
- Isoleucine
- Acetylarginine
- ADMA
- SDMA
- Asparagine
- Citrulline
- Cysteine
- Glutamine
- Hippurate
- Histidine
- Homocysteine
- Taurine

Dietary choline metabolisms (6)

Trimethylamine N-oxide	TMAO
Choline	Choline
L-carnitine	AC-C0
Betaine	betaine
Gamma-Butyrobetaine	γ-Bb
Acetyl-carnitine	AC-C2

Short chain fatty acids (6)

- Acetate
- Propionate
- Butyrate
- Isobutyrate
- Valerate
- Isovalerate

<u>The pipeline of analyses were standardized as follow :</u>