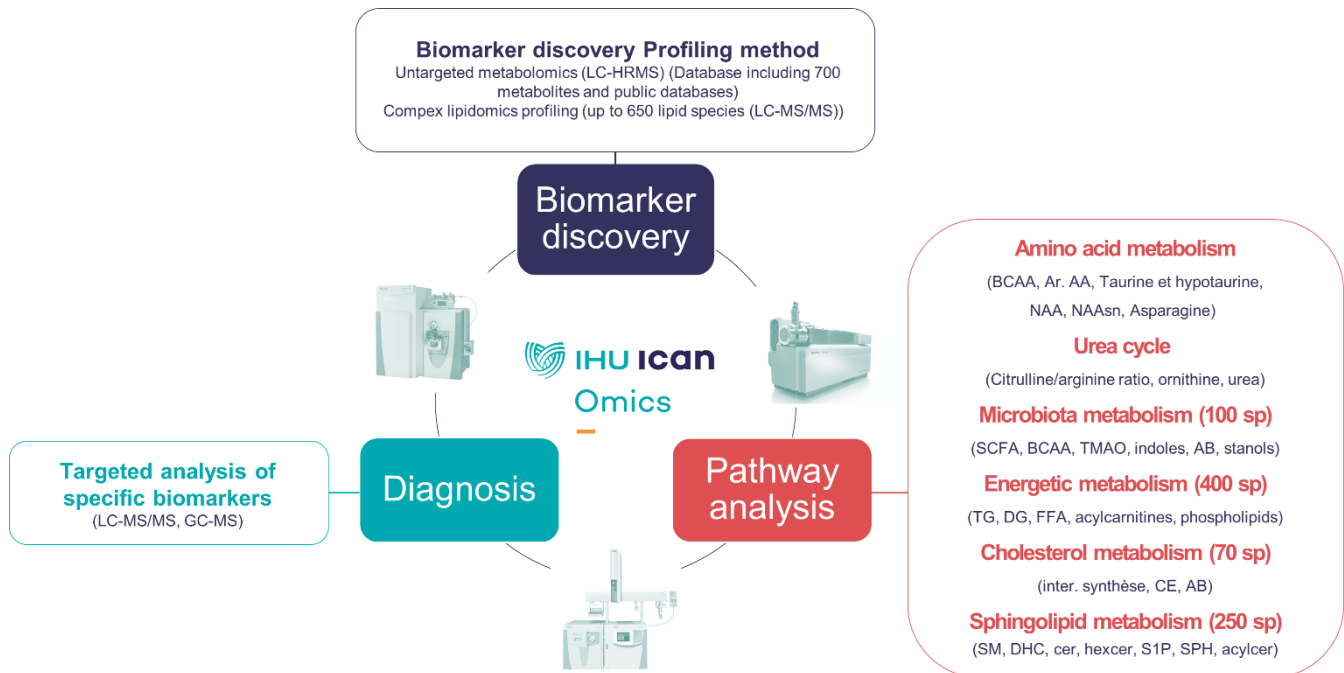


Several approaches were proposed by ICAN Omics metabolomics:

- **Untargeted approach 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

<b>Aminoacid metabolisms (41)</b>	<b>Tryptophan-kynurenine metabolisms (21)</b>	<b>Organic acids (34)</b>	<b>Indoles (9)</b>
Alanine	2-aminoadipic acid	2-hydroxyoctanoate	5-methoxyindoleacetate
Acetyl-Glycine	3-Hydroxykynurenine	Lactate	5-Hydroxyindoleacetate (5-HIAA)
Arginine	3-Hydroxytryptophane	2-OH-butyrate	IndoxylSulfate
Asparagine	3-Indolepropionate	Adipate	3-Indolebutyric acid
Aspartate	Anthranilic acid	Succinate	3-Indolepropionic acid
Citrulline	Hydroxyanthranilic acid	Aconitate (Cis&trans)	Indole-3-carboxylic acid
Creatine	Indole-3-acetate	Citrate-Isocitrate	Indole-3-pyruvic acid
Creatinine	Indole-3-lactate	Fumarate	Indole-3-carbaldehyde
Methionine-Sulfoxide	Kynurenic acid	Hippurate	Indole-3-acetaldehyde
Glutamic acid	Kynurenine	Homovanillate	
Glycine	Melatonin	Hydroxyhippurate	
Histidine	N-acetyl-tryptophane	Pipecolate	
Homocysteine	Niacinamide (B3)	Suberate	
Isoleucine	Nicotinic acid (B3)	Urocanate	
Kynurenine	N-methylnicotinamide	4-Aminobutanoate	<b>B-vitamins (10)</b>
Leucine	Picolinic acid	4-Acetamidobutanoate	6-Methylnicotinamide
Glutamine	Quinaldic	2-Hydroxy-3-methylbutyric acid	Pyridoxic acid (Vit B6)
Lysine	Quinolinic acid	3-Hydroxy-3-methylbutyric acid	Ascorbic acid (Vit C.)
Proline	Serotonin	2-Hydroxyhexanoic acid	N-methylnicotinamide
Serine	Tryptophan	Malate	p-aminobenzoic acid (Vit B10)
Methionine	Xanthurenic acid	4-Hydroxybenzoate	Pantothenic acid (B5)
N-acetyl-alanine		3-Hydroxybenzoate	Mandelate
N-acetyl-glutamate	<b>Purine and pyrimidine metabolisms (22)</b>	4-Acetamidobutanoate	Pyridoxal (Vit B6)
N-acetyl-tyrosine	1-Methyladenosine	2-Oxoglutarate	Pyridoxamine (Vit B6)
N-Acetyl-L-phenylalanine	3'-deoxyguanosine	Mandelate	Riboflavin (Vit B2)
5-Oxoproline	7-methylguanine	3-Phenylactic Acid	Thiamine (Vit B1)
N-alpha-acetyl-arginine	adenine	3-(4-Hydroxyphenyl)lactate	
Ornithine	cytidine	2-Methylhippuric acid	
Phenylalanine	Deoxycytidine	Ferulic acid	
Threonine-Homoserine	deoxyinosine	Alpha-Hydroxyhippuric Acid	
Tryptophan	deoxyuridine	Syringic acid	
Tyrosine	Guanine	Glutarate	
Valine	Guanosine	Pimelic acid	<b>Methylamine and polyamines metabolisms (9)</b>
N6,N6,N6-Trimethyl-L-lysine	Hypoxanthine	Hydrocinnamic Acid	2-PhenylEthylamine
N(pi)-Methyl-L-histidine	Inosine		Betaine
Hexanoylglycine	Methylcytidine	<b>Phenol, nutrient-related and exogene metabolites (8)</b>	Butyrobetaine
N-Acetyl-L-Leucine	Pseudouridine	4-Nitrophenol	Carnitine
2-Furoylglycine	thymidine	Trigonelline	Choline
Cinnamoylglycine	thymine	Dihydrocaffeic acid	TMAO
alpha-N-Phenylacetyl-L-glutamine	Uracil	Theobromine	N-Acetyl-putrescine
Total DMA (ADMA-SDMA)	Urate	Caffeine	Diethanolamine
	uridine	Acetaminophen	Acetylcholine
	3-Methyladenine	Vanillin	
	Xanthine	Metformin	

### 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

The pipeline of analyses were standardized as follow :

