Serum Metabolomics by GCMS as a Novel Diagnostic Approach for Disease

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Methods for Early Cancer Screening

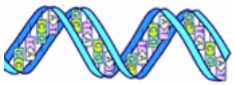
	Biochemical method (tumor markers)	Imaging method (CT, MRI, PET, endoscopy)
Detection rate	Low	High
Cost	Low	High
Operation	Easy	Difficult

A low-cost, easy-to-use, and high-sensitive cancer diagnosis method is desired.

Omics

DNA: Genomics

(25,000)

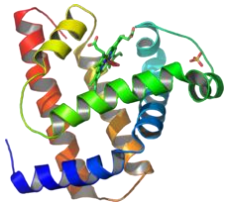


The large-scale study of genome finish reading the entire human genome in 2004

Factors regulating physiological Functions have been analyzed.

Protein: Proteomics

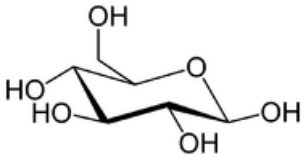
(1,000,000)



The large-scale study of proteins

Metabolite: Metabolomics

(4,000)



The systematic study of small molecule metabolite profiles

（Recent progress of mass spectrometer & analysis software）

Why metabolomics?

✓**Smaller numbers compared to genome and proteome.**

Human genome = about 25,000
Human proteome = about 1,000,000
Human metabolome = about 3,000-4,000

✓**Small molecules which have been detected using traditional analytical assays.**

Traditionally, single metabolite or class of small molecules have been well investigated in biochemical fields.
Metabolomics can globally analyze metabolites.

✓**Close to phenotype**

Alterations in genome, proteome do not always change phenotype due to homeostasis. However, metabolome is the unique chemical fingerprints that specific protein actions leave behind.

✓**No species-specificity**

Common analytical methods are available to samples from different species.

nature Vol 463|7 January 2010

OPINION

2020 visions

For the first issue of the new decade, *Nature* asked a selection of leading researchers and policy-makers where their fields will be ten years from now. We invited them to identify the key questions their disciplines face, the major roadblocks and the pressing next steps. Visit go.nature.com/htW8uM to respond and to add your vision.

Search

Peter Norvig*
Director of research at Google

Internet search as we know it is just one decade old; by 2020 it will have evolved far beyond its current bounds. Content will be a mix of text, speech, still and video images, histories of interactions with colleagues, friends, information sources and their automated proxies, and tracks of sensor readings from Global Positioning System devices, medical devices and other embedded sensors in our environment.

The majority of search queries will be spoken, not typed, and an experimental minority will be through direct monitoring of brain

ILLUSTRATIONS BY JESSE LEFKOWITZ

- Search Engines
- Microbiome
- Lasers
- Ecology
- Metabolomics

Metabolomics

Jeremy K. Nicholson
Head, Department of Surgery and Cancer, Imperial College London

The analysis of the chemical fingerprints left by metabolic processes has already started to play a crucial part in personalized medicine, particularly cancer therapy. This stems from the understanding that humans are metabolic superorganisms carrying the genomes of many symbiotic organisms, all of which can affect an individual's physiology. Human metabolism is heavily influenced by interactions between our own genes and the activities of gut microbes, as well as by diet and environmental stressors. The products of this metabolic interplay have a direct influence on susceptibility to disease.

Determining how the body's metabolic processes interact with those of gut microbes is a priority in the coming years, because many conditions, including ulcerative colitis, Crohn's disease, obesity, diabetes and autoimmune disorders, are linked to poor gut health and microbial imbalances. By 2020, personalized health care could involve doctors monitoring the metabolic activities of a patient's gut microbes and, possibly, modulating them therapeutically. The use of mathematical models to interpret metabolic data obtained using nuclear magnetic resonance spectroscopy and mass spectrometry will help us to understand the changing patterns of human disease on a global scale, and generate new targets for drug or nutritional interventions. ■

Omics research of blood for diagnosis

	Genomics (gene)	Proteomics (protein)	Metabolomics (metabolite)
Number of targets	≈ 25,000	≈ 100,000	≈ 4,000
Analysis	Laborious	Difficult	Easy
Health condition	Not reflect	Difficult to reflect	Easy to reflect

Metabolomics has potential to be utilized in early cancer diagnosis.

Advantage of a novel diagnosis method of cancer by metabolomics

1. **Easy-to-use and high generality**
2. **Analyze a lot of samples at a low cost in short times**
3. **Detect many metabolites using small amounts of blood (< 50 μL)**
4. **Perform diagnosis using disease-specific metabolite profile**

Metabolomics has a great potential of application to first screening of early cancer.

Metabolomics Research Center

Graduate school of medicine, Kobe university

The nation's first mass spectrometry center, which was specialized for clinical study, was established in 2008.
(The Integrated Center for Mass Spectrometry)

Kobe university hospital

SHIMADZU

Mass spectrometers

Shimadzu LCMS-IT-TOF

lipids

Shimadzu LC-8030

lipids (MRM methods)

Shimadzu LCMS-2010EV

amino acids

Agilent CE-TOF

ionic metabolites

Shimadzu GCMS-QP2010
QP2010 PLUS

Shimadzu
GCMS-QP2010 Ultra

Agilent
GCMS/MS

(MRM methods)

Shimadzu AXIMA-TOF²

MS imaging

organic acids, amino acids, fatty acids

For “Clinical Metabolomics”

Aims

STEP1: Establishment of metabolome analysis system using human samples (The methods for sample preparation, measurement and data analysis)

STEP2: Determination of metabolites changing in each disease (Statistical procedure)

STEP3: Investigation on applicability of changing metabolites as disease-specific biomarkers (The cause or consequence of diseases? etc…)

Flowchart of the cancer diagnosis method

Measuring object

Serum

Pretreatment

Patient

Analysis of metabolites

GC-MS

- ✓Amino acid
- ✓Organic acid
- ✓Fatty acid

metabolites profiling

Initial diagnosis

Imaging method (CT, MRI, PET, endoscopy)

Confirmed diagnosis

Experimental Methods

Fasting serum (50 µl)

With internal standard for quantitative calibration.

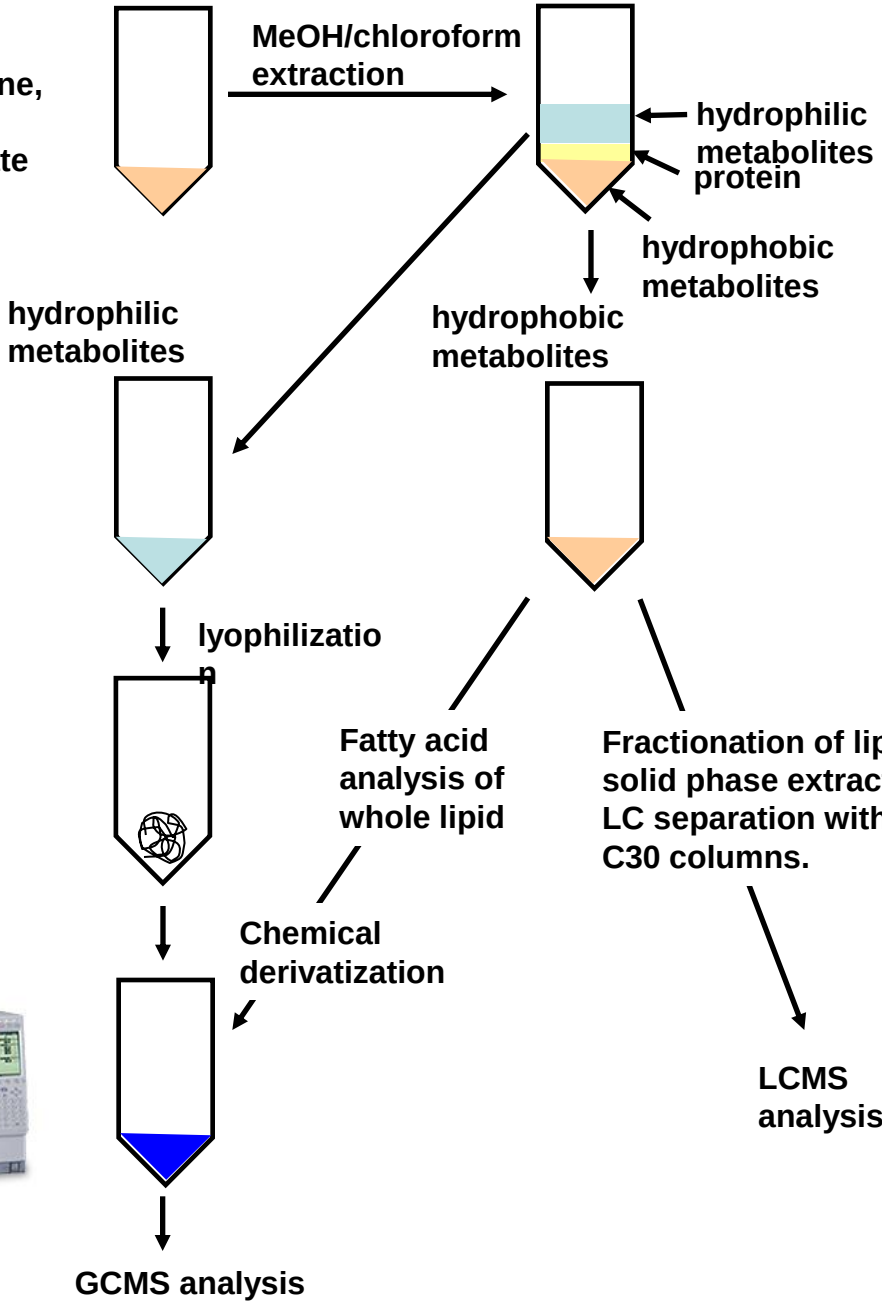
Extraction/separation of metabolites

Hydrophilic/hydrophobic metabolites were extracted by MeOH/$CHCl_3$ extraction.

GCMS analysis

After chemical derivatization (GCMS)

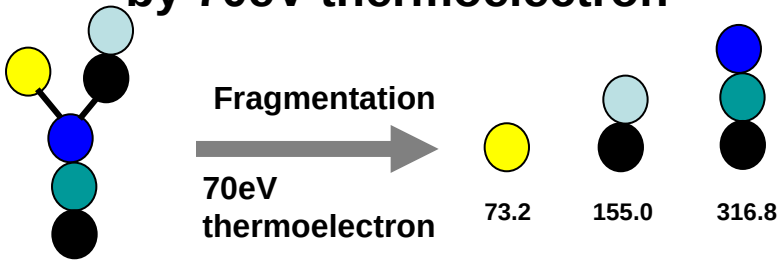
Data analysis



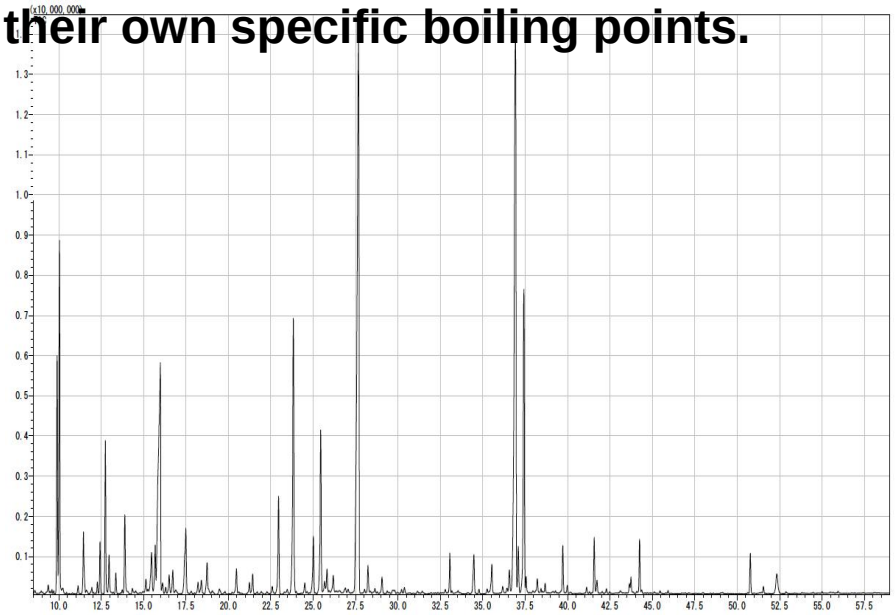
Metabolomics Analysis by GCMS

capilary column for metabolites separation in the colum oven

each metabolite is fragmented by 70eV thermoelectron

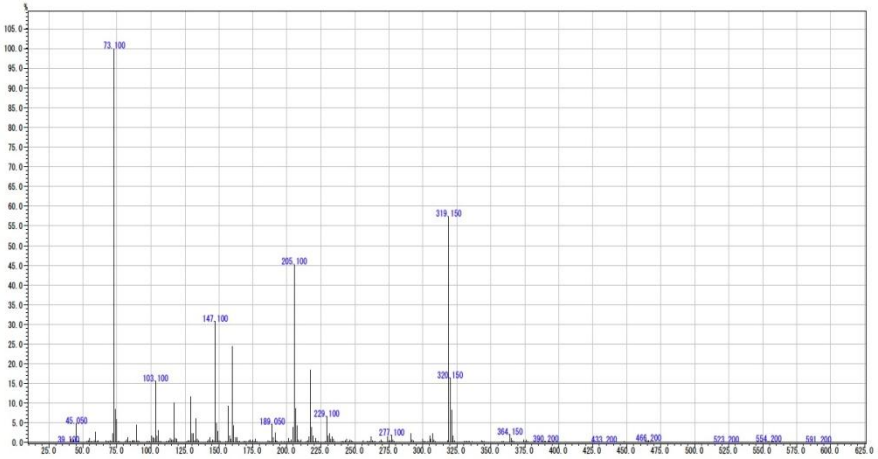


metabolites are efficiently separated at their own specific boiling points.

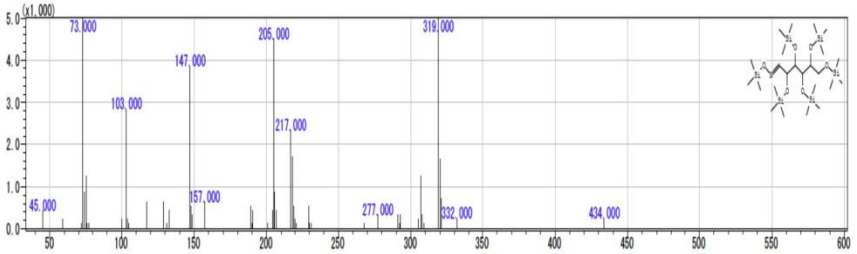


Column oven temp.
100ºC~

~320ºC



EI spectrum



Database spectrum

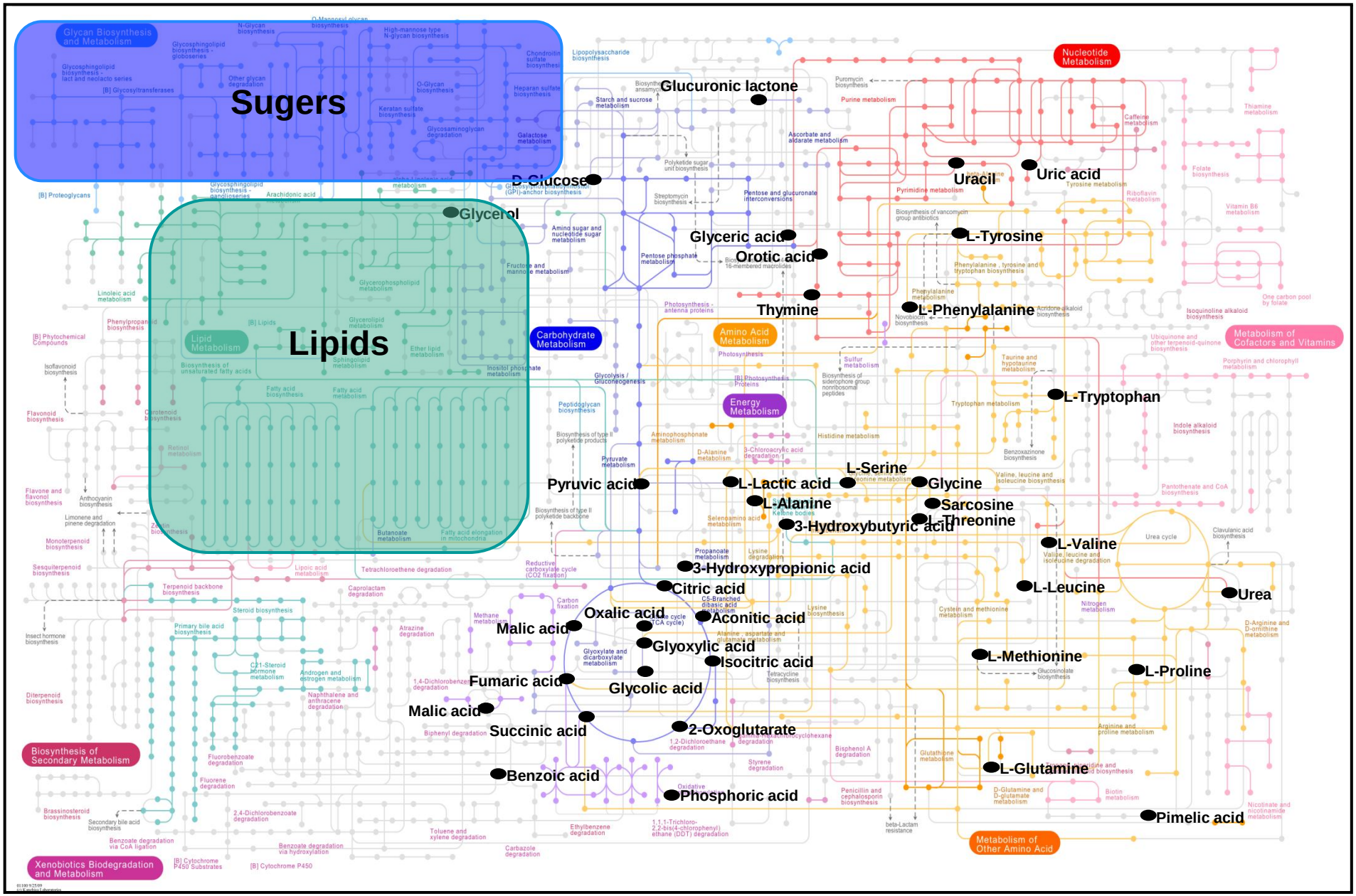
Metabolites database for identification by GC/MS

1	Boric acid	51	allo-Isoleucine	101	Cinnamic acid	151	Caffeine
2	Trichloroacetic acid	52	Phenylacetic acid	102	5-Hydroxy-2-furoic acid	152	Hydroxylysine (2 isomers)
3	Phenol	53	Maleic acid	103	Tiglylglycine	153	Methylcitric acid
4	Lactic acid	54	L-Proline	104	3-Methylcrotonylglycine	154	Vanilmandelic acid
5	2-Hydroxyisobutyric acid	55	2-Octenoic acid	105	Tiglylglycine	155	Sebacic acid
6	Caproic acid	56	Succinic acid	106	3-Hydroxybenzoic acid	156	Decadienedioic acid
7	Glycolic acid	57	Methylsuccinic acid	107	3-Methylcrotonylglycine	157	4-Hydroxyphenyllactic acid
8	L-Alanine	58	Glyceric acid	108	2-Hydroxyphenylacetic acid	158	Theophylline
9	L-Glycine	59	Fumaric acid	109	2-Hydroxyglutaric acid	159	L-Histidine
10	Glyoxylic acid	60	Uracil	110	Pimelic acid	160	3,4-Dihydroxymandelic acid
11	Oxalic acid	61	Citraconic acid	111	3-Hydroxy-3-methylglutaric acid	161	L-Tyrosine
12	2-Hydroxybutyric acid	62	Propionylglycine	112	3-Hydroxyphenylacetic acid	162	Indole-3-acetic acid
13	2-Furoic acid	63	L-Serine	113	L-Glutamic acid	163	Palmitoleic acid
14	Sarcosine	64	Acetylglycine	114	4-Hydroxybenzoic acid	164	Palmitic acid
15	3-Hydroxypropionic acid	65	Mevalonic lactone	115	2-Ketoglutaric acid	165	2-Hydroxysebacic acid
16	Pyruvic acid	66	Isobutyrylglycine	116	L-Phenylalanine	166	3-Hydroxysebacic acid
17	Valproic acid	67	2-propyl-3-hydroxy-pentanoic acid	117	4-Hydroxyphenylacetic acid	167	2-Hydroxyhippuric acid
18	4-Cresol	68	L-Threonine	118	Lauric acid	168	Dodecanedioic acid
19	3-Hydroxybutyric acid	69	Mesaconic acid	119	Tartaric acid	169	Naproxen
20	3-Hydroxyisobutyric acid	70	Glutaric acid	120	Hexanoylglycine	170	N-Acetyltyrosine
21	2-Hydroxyisovaleric acid	71	Thymine	121	2-Ketoglutaric acid	171	Uric acid
22	alpha-Aminobutyric acid	72	3-Methylglutaconic acid	122	N-Acetylaspartic acid	172	Margaric acid
23	2-Methyl-3-hydroxybutyric acid	73	3-Methylglutaric acid	123	Glutaconic acid	173	3,6-Epoxydodecanedioic acid
24	Malonic acid	74	Propionylglycine	124	N-Acetylaspartic acid	174	Indolelactic acid
25	beta-Aminoisobutyric acid	75	Isobutyrylglycine	125	Asparagine	175	Stearic acid
26	3-Hydroxyisovaleric acid	76	2-Deoxytetronic acid	126	2-Hydroxyadipic acid	176	L-Tryptophan
27	2-Keto-isovaleric acid	77	3-Methylglutaconic acid(E)	127	Octenedioic acid	177	3-hydroxydodecanedioic acid
28	Methylmalonic acid	78	Glutaconic acid	128	3-Hydroxyadipic acid	178	Chloramphenicol
29	L-Valine	79	Succinylacetone	129	Suberic acid		
30	Ethylhydracrylic acid	80	Decanoic acid	130	Lysine		
31	Urea	81	3-Methylglutaconic acid(Z)	131	2-Keto-adipic		
32	4-Hydroxybutyric acid	82	2-Propyl-5-hydroxy-pentanoic acid	132	alpha-Aminoadipic acid		
33	2-Hydroxyisocaproic acid	83	Citramalic acid	133	Tricarballylic acid		
34	3-Hydroxyvaleric acid	84	Mandelic acid	134	Glutaconic acid		
35	D,L-Norvaline	85	Isovalerylglycine	135	Aconitic acid		
36	Acetoacetic acid	86	Malic acid	136	Orotic acid		
37	2-Hydroxy-3-Methylvaleric acid	87	Adipic acid	137	3-Methoxy-4-hydroxybenzoic acid		
38	Benzoic acid	88	Phenyllactic acid	138	Homovanillic acid		
39	Acetoacetic acid	89	p-Nitrophenol	139	L-Glutamine		
40	Octanoic acid	90	Isovalerylglycine	140	Azelaic acid		
41	Cyclohexanediol	91	2-Hexenedioic acid	141	Hippuric acid		
42	2-Methyl-3-hydroxyvaleric acid	92	Aspartic acid	142	Isocitric acid		
43	2-Propyl hydroxyglutaric acid	93	L-Methionine	143	Citric acid		
44	L-Leucine	94	5-Oxoproline	144	Glucuronoic lactone		
45	Glycerol	95	Thiodiglycolic acid	145	Hippuric acid		
46	Acetylglycine	96	4-Hydroxyproline	146	Homogentisic acid		
47	Phosphoric acid	97	3-Methyladipic acid	147	Myristic acid		
48	Ethylmalonic acid	98	Acetylsalicylic acid	148	Glucuronoic lactone		
49	2-Ketoisocaproic acid	99	7-Hydroxyoctanoic acid	149	Methylcitric acid		
50	L-Isoluecine	100	2-Propyl-glutaric acid	150	3-(3-Hydroxyphenyl)-3-hydroxypropionic acid		

Currently about 500 metabolites In our database

- Amino acids
- Other organic acids
- Other organic acids(Fatty Acids)
- Alcohols
- Ketones
- Nucleosides
- Carbohydrates
- Heterocyclic molecules
- Inorganic compounds

Metabolites detected in serum by GC/MS



Toward the realization of the system in the health examination

There are four steps to automate in the future.

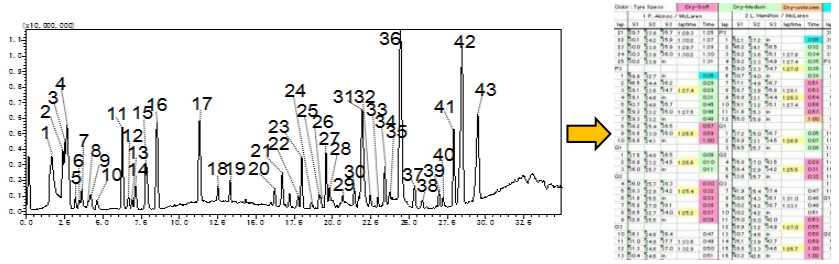
1. Automation of pretreatment

The expert's pretreatment process will be replicated.

2. MS analysis

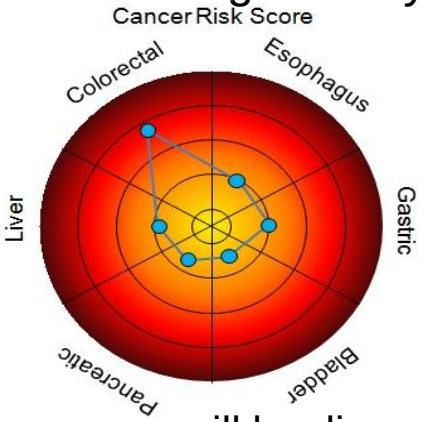
Downsizing and one-touch operation will be enabled.

3. Automation of MS data processing



Peak areas of mass chromatograms will be measured automatically.

4. Automatic diagnosis system



Multiple cancers will be diagnosed at same time by cancer profiling.

Future Goal

Biochemical Diagnosis

Normal level

✓ Inflammatory markers (GOT, GPT, etc)

✓ Tumor markers (AFP, PIVKA-II)

High level

✓ Metabolome analysis

Diagnostic Imaging

Normal level

✓ X-ray ✓ Echo

High level

✓ CT ✓ PET ✓ MRI

Integrated diagnostics

For Early diagnosis, Prognosis, Therapy evaluation