

# **Development of an Assay for Dietary and Exposome Measurements for Precision Medicine**

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# Outline

- **Brief Introduction to Precision Medicine**
- **Metabolomics, IEMs, Nutrition and Precision Medicine**
- **Integrating Multi-Omics for Precision Medicine – the Molecular You Experience**
- **Lessons Learned**
- **Metabolomics Kits for Nutrition/Exposure Measurement**

# Precision Medicine vs. Personalized Medicine

- **Personalized Medicine:** Developing treatments and preventative strategies that are unique to a specific individual
- **Precision Medicine:** Providing the right diagnosis and the right treatment to all by giving the right drug at the right dose to the right patient at the right time
- *“Medicine has always been very personal, but it hasn’t always been very precise”*

# Why Metabolomics for Precision Medicine?

- The genome tells us what **might** happen; the metabolome tells us what **is** happening
- Metabolites are easy to change (diet, supplements, drugs), genes are not
- Metabolomics is fast, cheap, quantitative, comprehensive and the data is easy to interpret
- Lots of very useful metabolite biomarkers

# Metabolomics is Moving to the Bedside

- Number of “approved” tests arising from **Metabolomics**/Clinical Chem. – **327**
- Number of “approved” tests arising from or using **Genomics** – **130**
- Number of “approved” single **Protein** tests (ELISA) – **108**
- Number of “approved” tests arising from or using **Transcriptomics** – **5**
- Number of “approved” tests arising from or using **Proteomics** - **1**

# Common Metabolite Biomarkers

- **Glucose ( $> 6.1$  mM – diabetes)**
- **Creatinine ( $> 110$   $\mu$ M – kidney damage)**
- **Cholesterol ( $> 5.2$  mM – CVD risk)**
- **Triglycerides ( $> 1.8$  mM – CVD risk)**
- **Uric acid ( $> 0.5$  mM – gout)**
- **T<sub>4</sub> Thyroxine ( $> 160$  nM – hyperthyroidism)**
- **T<sub>4</sub> Thyroxine ( $< 60$  nM – hypothyroidism)**
- **Phe/Tyr Ratio ( $> 2.5$  – PKU)**

# Precision Medicine Example: Newborn Screening of IEMs



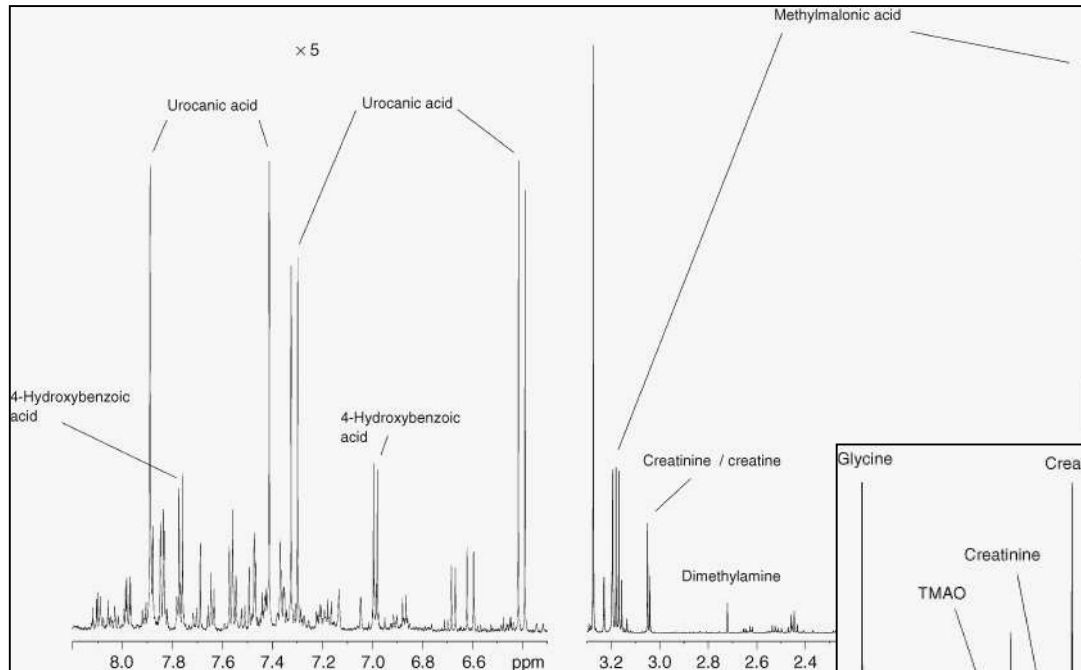
**Almost everyone <25 yrs old has had a metabolomic test**

# Why Newborn Screening of IEMs is a Perfect Example for Precision Medicine & Precision Nutrition

- Offers precise, personalized diagnoses (and even prognoses)
- Catches the condition early so that *customized diets, supplements*, lifestyle changes, or drugs can be developed, delivered or prescribed
- Can play a key role in prevention, treatment or mitigation of disease or symptoms
- Constant monitoring of the *metabolic phenotype* using precise chemical methods enables *precision nutrition*



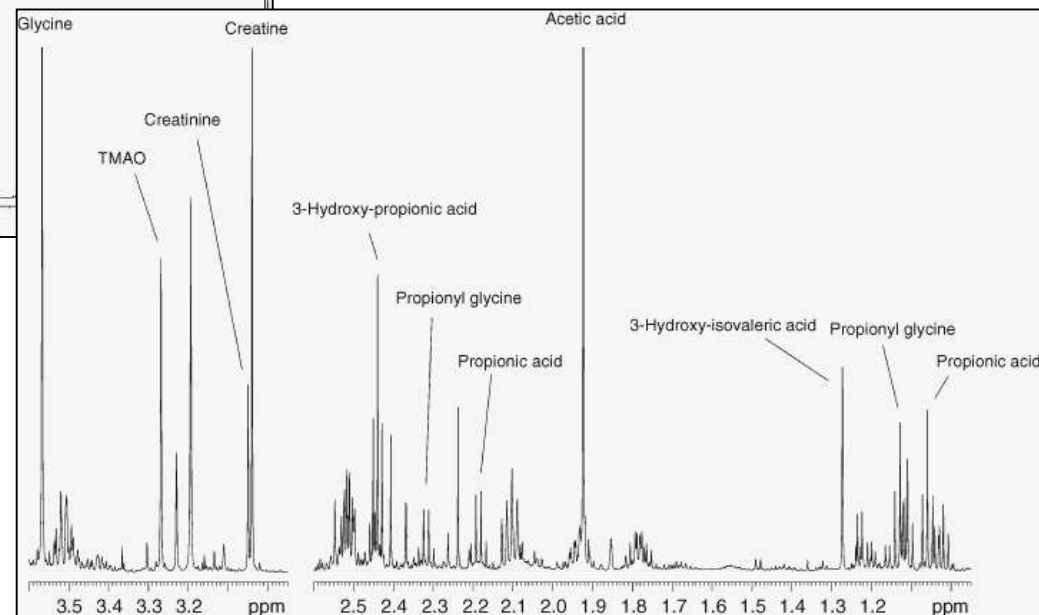
# Detecting IEMs by NMR-based Metabolomics



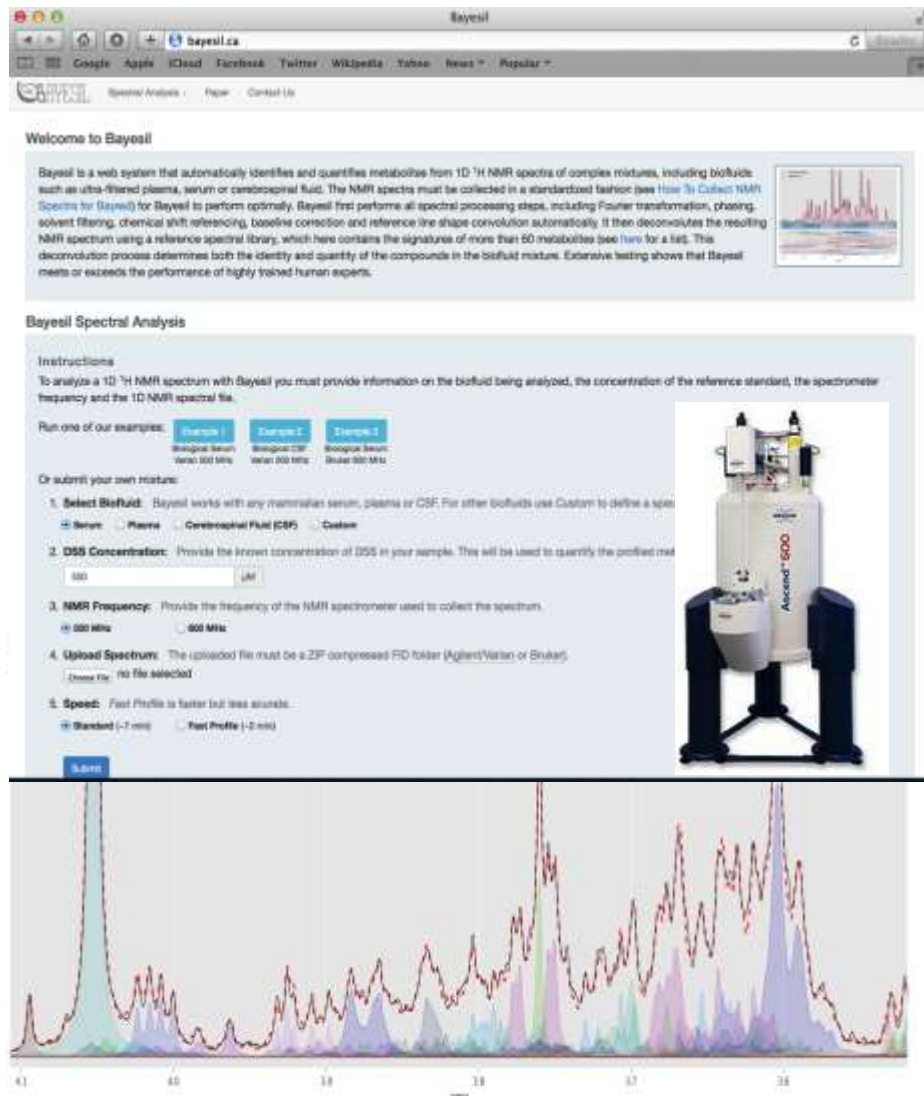
Methylmalonic aciduria



Propionic aciduria



# The Latest in NMR Screening



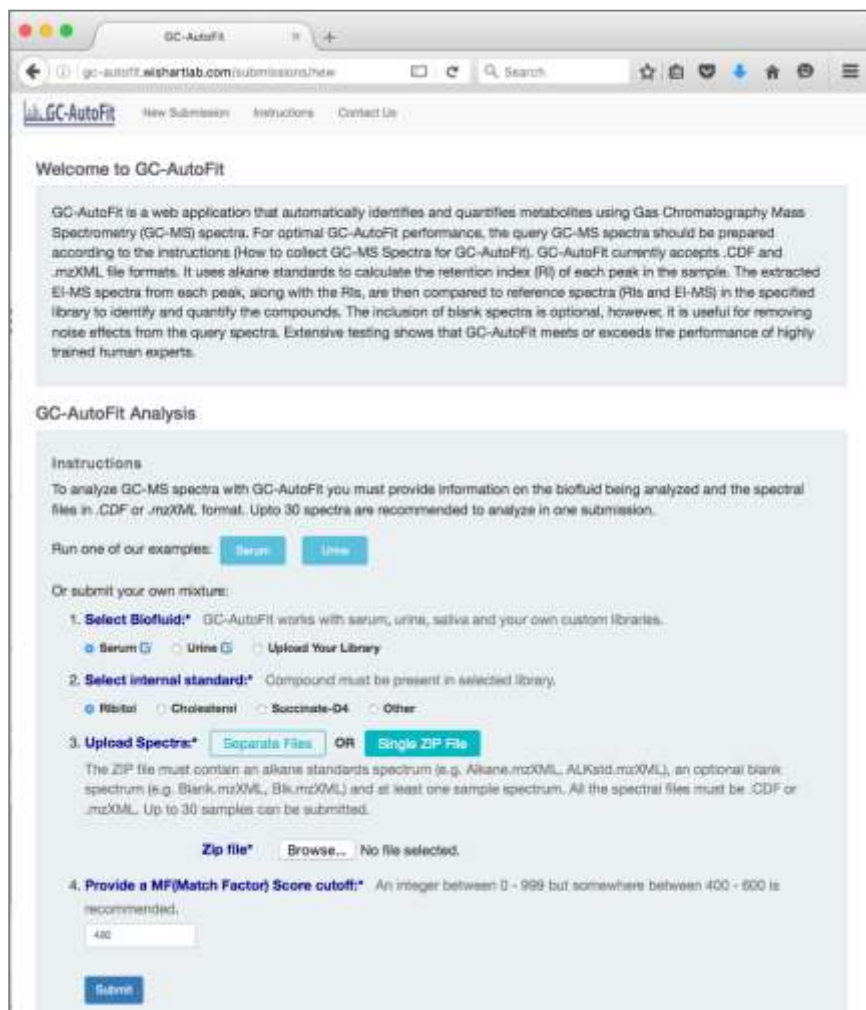
- **Bayesil - Web-based tool for automated NMR spectral profiling**
- **Identifies & quantifies all metabolites in an NMR spectrum**
- **Uses probabilistic graphical models (PGM) – like HMMs**
- **Fits shift & peak intensity similar to the way humans perform fitting & pattern finding**
- **Works for CSF, serum, saliva, urine – up to 90 cmpds ID'd**
- **Fully automated phasing, referencing, water removal, baseline correction, identification & quantification**
- **150-200 sec/sample**

<http://bayesil.ca>

# Automated IEM Screening via NMR-based Metabolomics

- 14 propionic acidemia
- 11 methylmalonic aciduria
- 11 cystinuria
- 6 alkaptonuria
- 4 glutaric aciduria I
- 3 pyruvate decarboxylase deficiency
- 3 ketosis
- 3 Hartnup disorder
- 3 cystinosis
- 3 neuroblastoma
- 3 phenylketonuria
- 3 glycerol kinase deficiency
- 3 HMG CoA lyase deficiency
- 2 carbamoyl PO<sub>4</sub> synthetase deficiency
- **96% sensitivity and 100% specificity in ID of abnormal from normal by metabolite concentrations**
- **95.5% sensitivity and 92.4% specificity in ID of specific disease or specific condition by characteristic metabolite concentrations**

# The Latest in GC-MS Screening



The screenshot shows the GC-AutoFit web application interface. At the top, there's a navigation bar with 'GC-AutoFit' and links for 'New Submission', 'Instructions', and 'Contact Us'. Below this, a 'Welcome to GC-AutoFit' message is displayed. The main content area is titled 'GC-AutoFit Analysis' and contains 'Instructions' on how to use the application. It mentions that the application automatically identifies and quantifies metabolites using Gas Chromatography Mass Spectrometry (GC-MS) spectra. It also states that the query GC-MS spectra should be prepared according to the instructions (How to collect GC-MS Spectra for GC-AutoFit). The application currently accepts .CDF and .mzXML file formats. It uses alkane standards to calculate the retention index (RI) of each peak in the sample. The extracted EI-MS spectra from each peak, along with the RIs, are then compared to reference spectra (RIs and EI-MS) in the specified library to identify and quantify the compounds. The inclusion of blank spectra is optional, however, it is useful for removing noise effects from the query spectra. Extensive testing shows that GC-AutoFit meets or exceeds the performance of highly trained human experts.

Below the instructions, there are two buttons: 'Serum' and 'Urine'. Underneath, it says 'Or submit your own mixture:'.

1. **Select Biofluid:**\* GC-AutoFit works with serum, urine, saliva and your own custom libraries.  
☒ Serum ☐ Urine ☐ Upload Your Library

2. **Select internal standard:**\* Compound must be present in selected library.  
☒ Ribitol ☐ Cholesterol ☐ Succinate-D4 ☐ Other

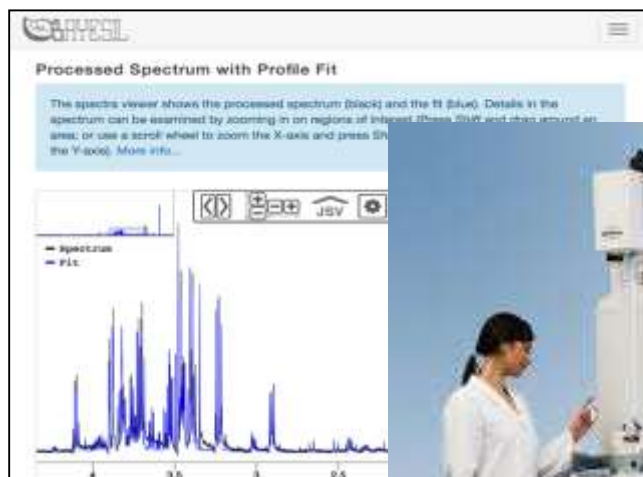
3. **Upload Spectra:**\*  OR   
The ZIP file must contain: an alkane standards spectrum (e.g. Alkane.mzXML, ALKstd.mzXML), an optional blank spectrum (e.g. Blank.mzXML, Bli.mzXML) and at least one sample spectrum. All the spectral files must be .CDF or .mzXML. Up to 30 samples can be submitted.  
Zip file\*  No file selected.

4. **Provide a MF(Match Factor) Score cutoff:**\* An integer between 0 - 999 but somewhere between 400 - 600 is recommended.

- **GC-AutoFit - Fully automated GC-MS compound ID and quantification of urine**
- **Requires 3 spectra (sample, blank, alkane standards)**
- **Performs auto-alignment, peak ID, peak integration and concentration calculation**
- **Accepts NetCDF or mzXML files**
- **60 sec per spectrum**
- **75 cmpds ID' d and quantified, in urine with >99% accuracy**
- **Up to 110 cmpds in Nov. 2017**
- **Now being validated in clinical testing labs for IEM screening and nutrient monitoring**

<http://gc-autofit.wishartlab.com>

# Moving from the Lab to the Clinic with Kits



**NMR Metabolomics Kit**



**GC-MS Metabolomics Kit**

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# Precision Medicine Needs Multi-Omics Measurements

- **Metabolomics** measures the chemical phenotype, diet and exposures
- **Proteomics** measures cellular functions and cellular activity
- **Microbiomics** measures microbial activity in the gut and diet
- **Genomics** measures genetic risk through genes and SNPs
- Together they give a “systems” view



# Combining Multiple Omics Measures for Precision Health



- **Metabolomics**
- **Microbiomics**
- **Proteomics**
- **Genomics**

[www.molecularyou.com](http://www.molecularyou.com)

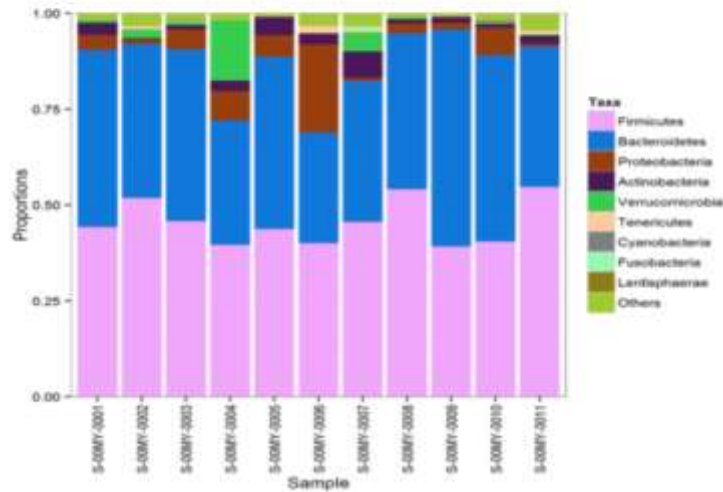




# Quantitative Omics

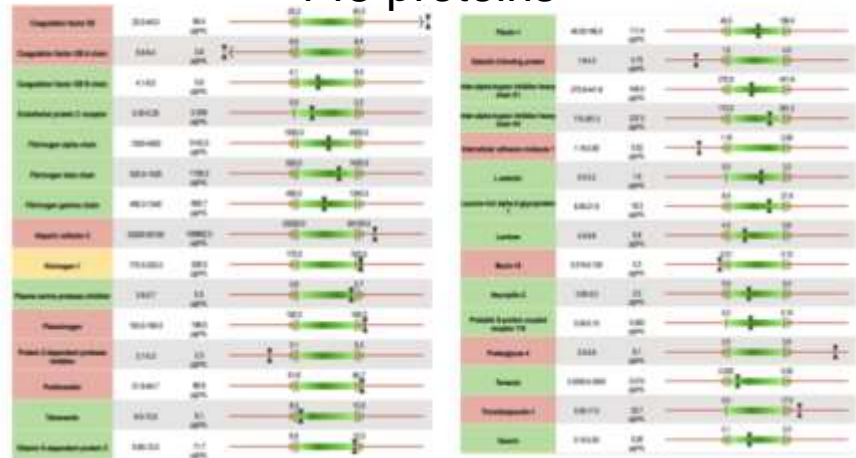
## Quantitative Microbiomics

21 microbial taxa



## Quantitative Proteomics

143 proteins



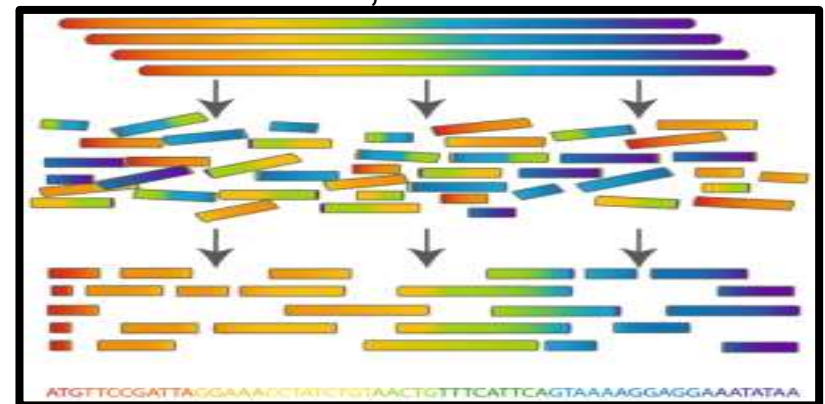
## Quantitative Metabolomics

186 cmpds

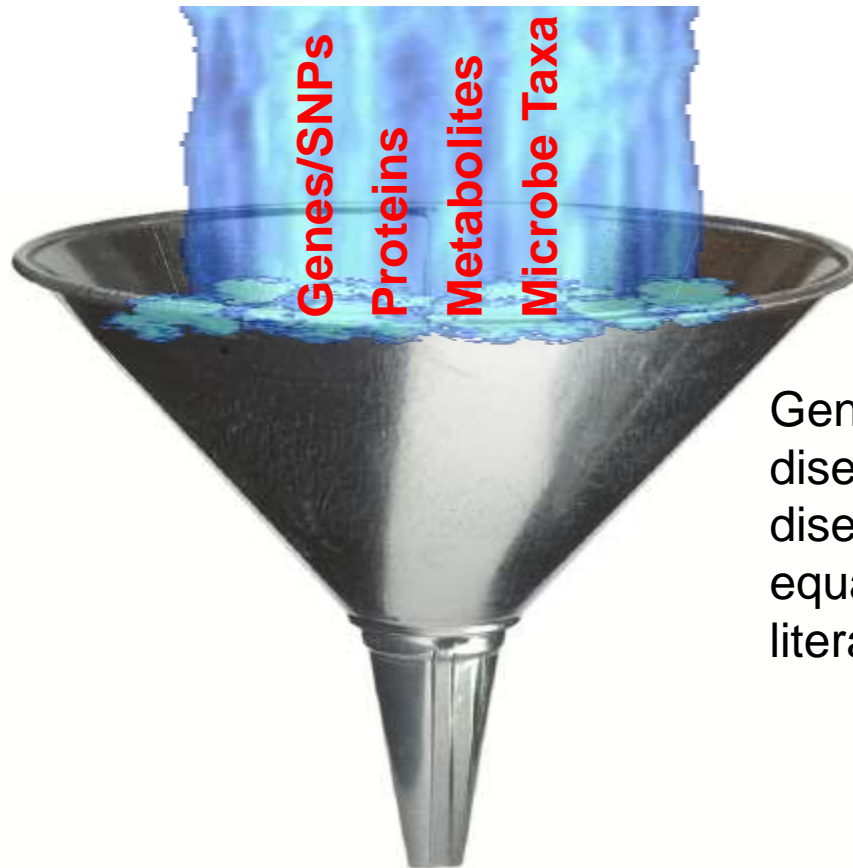


## Quantitative Genomics

700,000 SNPs





# Biomarker Integration



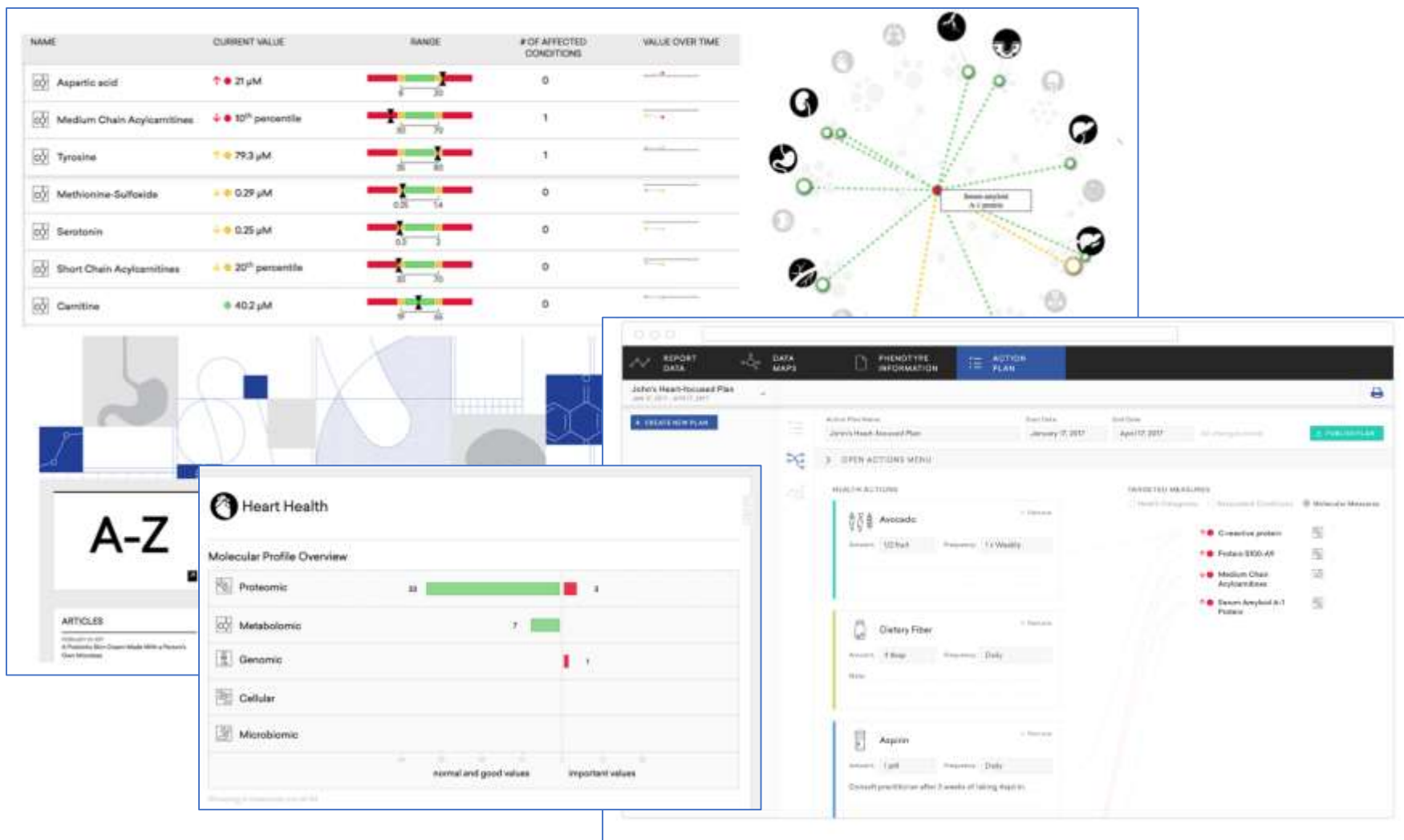
Gender, age, disease prevalence  
disease incidence, age-related  
disease prevalence, log-regression  
equations, platform weightings,  
literature evidence, etc.

**Molecularly-derived  
disease risk score**

# Data-Disease-Physician Integration

Health Category	Health Term	#
<b>Metabolic Health - ★★</b>    	<b>Diabetes</b> <ul style="list-style-type: none"> <li>• Apolipoprotein C-II - <i>high</i></li> <li>• Kininogen-1 - <i>moderately high</i></li> <li>• Thrombospondin-1 - <i>high</i></li> <li>• Vitronectin - <i>moderately high</i></li> <li>• Glucose - <i>high</i></li> <li>• Leucine - <i>moderately high</i></li> <li>• Se - <i>moderately low</i></li> <li>• rs5443 - <i>Bad</i> - 2.4</li> <li>• rs3184504 - <i>Bad</i> - 2.0</li> <li>• rs1042522 - <i>Bad</i> - 2.0</li> <li>• rs560887 - <i>Bad</i> - 2.0</li> </ul>	7.0
	<b>Insulin resistance</b> <ul style="list-style-type: none"> <li>• Apolipoprotein B-100 - <i>high</i></li> <li>• Thrombospondin-1 - <i>high</i></li> <li>• Glucose - <i>high</i></li> <li>• Leucine - <i>moderately high</i></li> <li>• rs5443 - <i>Bad</i> - 2.4</li> </ul>	4.0
	<b>Obesity</b> <ul style="list-style-type: none"> <li>• Insulin-like growth factor binding protein acid labile subunit - <i>high</i></li> <li>• Insulin-like growth factor-binding protein complex acid labile subunit - <i>high</i></li> <li>• Thrombospondin-1 - <i>high</i></li> <li>• rs5443 - <i>Bad</i> - 2.4</li> </ul>	3.5
	<b>Pre-diabetes</b> <ul style="list-style-type: none"> <li>• Glucose - <i>high</i></li> <li>• Leucine - <i>moderately high</i></li> <li>• rs560887 - <i>Bad</i> - 2.0</li> </ul>	2.0
	<b>High BMI</b> <ul style="list-style-type: none"> <li>• Insulin-like growth factor binding protein acid labile subunit - <i>high</i></li> <li>• Insulin-like growth factor-binding protein complex acid labile subunit - <i>high</i></li> </ul>	2.0

# Data Tracking and Action Plans



[www.molecularyou.com](http://www.molecularyou.com)

# Action Plans

- **Focus on drug, diet and lifestyle recommendations**
- **Guided by nutritionist and physician input, customized to meet each patient's 'omic profile**
- **Builds on 1000's of pharmaco-metabolomic, pharmco-proteomic, nutri-metabolomic, nutri-proteomic and food composition associations compiled from the literature**

# Some Interesting Lessons

- **Patient-physician interactions are key**
- **Metabolomics is the most informative, genomics and microbiomics the least**
- **Patients are focused on fixing what's abnormal via diet, supplements and lifestyle changes**
- **Physicians are focused on fixing what's abnormal via drugs and medications**
- **Few users are truly sick, most are healthy and mostly focused on wellness and prevention**
- **Metabolites appear to be the most responsive to lifestyle and dietary changes – this is very empowering to patients**



# More Lessons...

- Both physicians and patients wanted more “high value” metabolites measured (i.e. those associated with common illnesses) as well as an “exposome” assay that measured diet, nutrient status and possible toxic exposures
- MYCo wanted cheaper, customized MS-based, targeted metabolomic assays
- MYCo & physicians wanted *precision nutrition* for better patient “action plans”

# Back to the Drawing Board...



**Commercial Kit**



**Custom Kit**

(The Metabolomics Innovation Centre  
(TMIC))



# Precision Metabolomics Kits

- **Old kit – 186 metabolites (lipids, lysoPCs, acylcarnitines, amino acids, biogenic amines) -- \$65/sample**
- **New kit #1 – 145 metabolites (organic acids, amino acids, common medical markers) for serum -- \$12/sample**
- **New kit #2 – 96 diet & exposure compounds + 21 metals for serum and urine -- \$35/sample**

# Kit #2 Compound Panels

Vitamins	Miscellaneous	Organic Acids
Vitamin B1	Sarcosine	Aminobutyric acid
Vitamin B2	Taurine	HPHPA
Vitamin B3	Trimethylamine N-oxide	Hippuric acid
Vitamin B5	Alpha-aminoadipic acid	5-Hydroxyindole-3-acetic acid
Vitamin B6	Carnosine	Vanillic acid
Vitamin B7	Phosphoserine	Ferulic acid
Vitamin B9	Cystathionine	Gallic acid
Vitamin B12	Proline-Betaine	Caffeic acid
Vitamin C	Trigonelline	Syringic acid
Choline	Acetylcarnitine	m-Coumaric acid
Vitamin A	Priopionylcarnitine	Salicylic acid
Vitamin D2	Carnitine	4-Pyridoxic acid
Vitamin D3	Bisphenol A	Hydroxyphenylacetic acid
25-Hydroxyvitamin D3	Triclosan	3-Hydroxyisovaleric acid
Vitamin E	Ethylsulfate	Chlorogenic acid
Vitamin K1	Diethylsulfate	3-Phenoxybenzoic acid
Vitamin K2	Cotinine	Perfluro-octanoic acid

■ done ■ in progress

# Kit #2 Cmpd Panels (Contd.)

Amino Acids & Derivatives		Fatty Acids	Metals	
Alanine	Valine	Lauric acid	Aluminium	Magnesium
Arginine	Tyrosine	Myristic acid	Antimony	Manganese
Asparagine	Methylhistidine	Myristoleic acid	Arsenic	Nickel
Aspartate	Homocysteine	Palmitic acid	Barium	Phosphorus
Citrulline	Hydroxyproline	Palmitoleic acid	Boron	Potassium
Glutamate	Ornithine	cis-10-Heptadecenoic acid	Cadmium	Selenium
Glutamine	Phenylalanine	Stearic acid	Calcium	Sodium
Glycine	Proline	Oleic acid	Chromium	Strontium
Histidine	Serine	Linoleic acid	Cobalt	Zinc
Isoleucine	Threonine	Linolenic acid	Copper	Iron
Leucine	Tryptophan	cis-10-Nonadecenoic acid	Lead	
Lysine	Beta-alanine	cis-8,11,14-Eicosatrienoic acid		
Methionine	Anserine	Arachidonic acid		
	Cystine	Eicosapentaenoic acid		
	Cysteine	Behenic acid		
		Docosapentaenoic acid		
		Docosahexaenoic acid	■ done	■ in progress
		Lignoceric acid		

# Sample Food Biomarkers in New MS Exposome/Diet Kit

Marker	Food	Marker	Food
Beta-alanine	Beef	Hippuric acid	Polyphenolic food
4-Hydroxyproline	Beef	Proline betaine	Citrus fruit
TMAO	Fish (cold water)	Citrulline	Watermelon
3-Met-Histidine	Chicken	Caffeic acid	Wine, herbs
Anserine	Chicken	Trigonelline	Coffee
Carnosine	General meat	Chlorogenic acid	Coffee
Acetylcarnitine	General meat	Beta carotene	Vegetables/Fruit
Propionylcarnitine	General meat	OH-phenylacetate	Vegetables
EPA	Oily fish	Gallic acid	Wine, grapes
DHA	Oily fish	Ferulic acid	Rye bread

# Analytical Methods

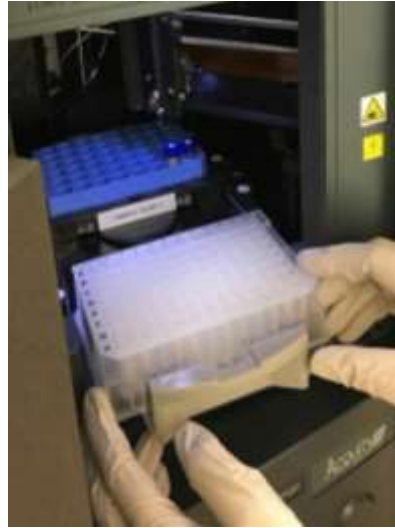
- **ICP-MS (Metal ions) – PerkinElmer Nexion 350X**
  - **Multiple QC metal ion standards**
- **Metabolomics Kit (LC-MS) – AB Sciex Qtrap 4000/5500, Standard HPLC**
  - **96-well plate assay**
  - **Standards (isotope-labeled, calibrants, QC)**
  - **Derivatization reagents (PITC and 3-NPH)**
  - **Universal C18 Column**
  - **3 LC-MS Runs (Amino acids/Amines, Organic acids/Fatty acids, Vitamins)**

# Experimental Set-up

LCMS



Sample Preparation

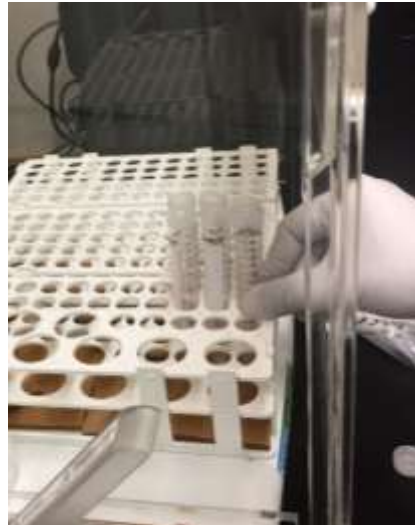


Autosampler

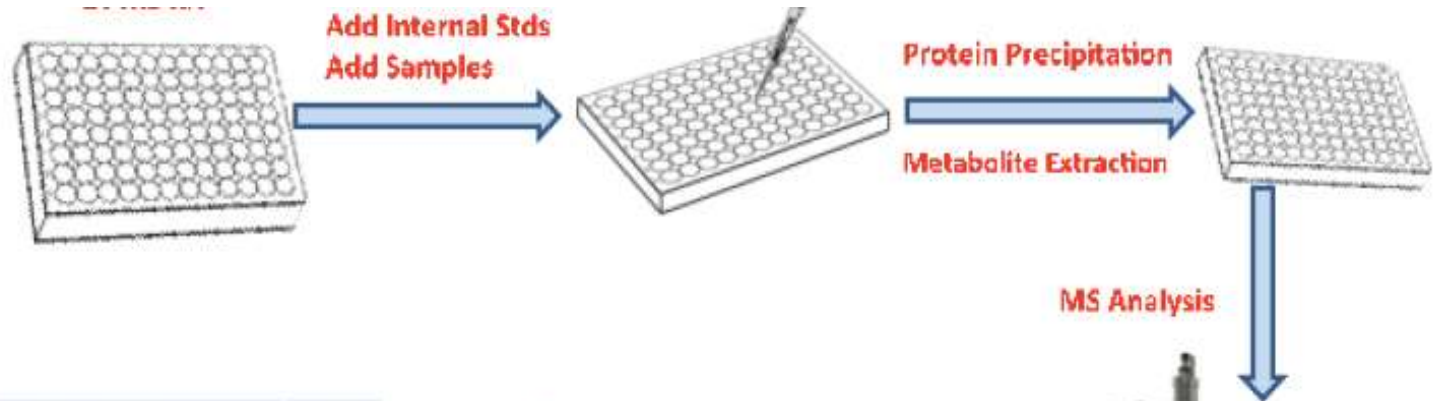


Sample Analysis

ICPMS

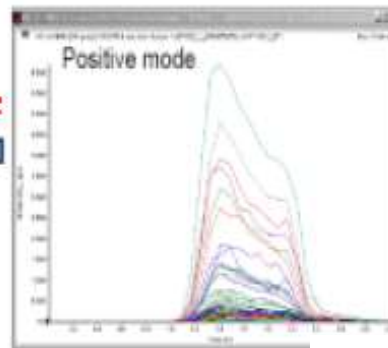


# LC-MS Experimental Protocol

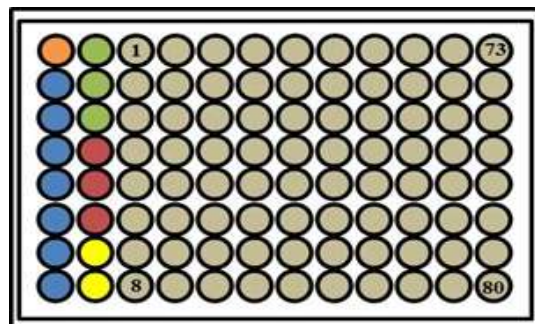


Asymmetric Dimethylarginine	0.56
Aminoadipic acid	2.14
Delta-Hydroxybutyrate	50
Betaine	12.5
Milrinone	15
Carnitine	0.05
Choline	0.2
Creatinine	0.05
Folate	0.025
Formate	24.5
Glucose	3450

MS-AutoFit



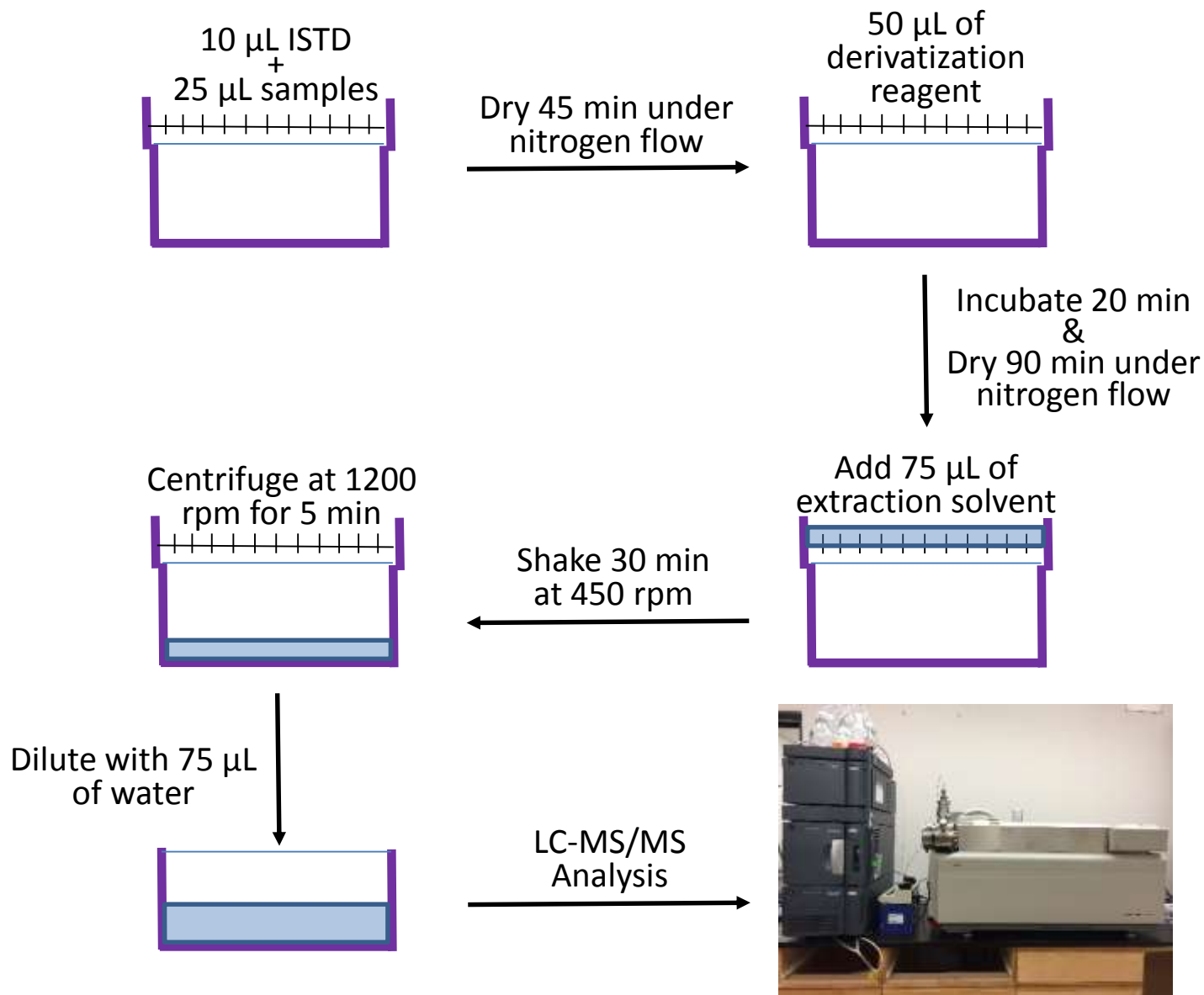
MS Analysis



- Reagent Blank
- Calibration Standards
- QC Standards
- NIST SRM-1950 Reference
- QC - Pooled Human Serum
- Samples



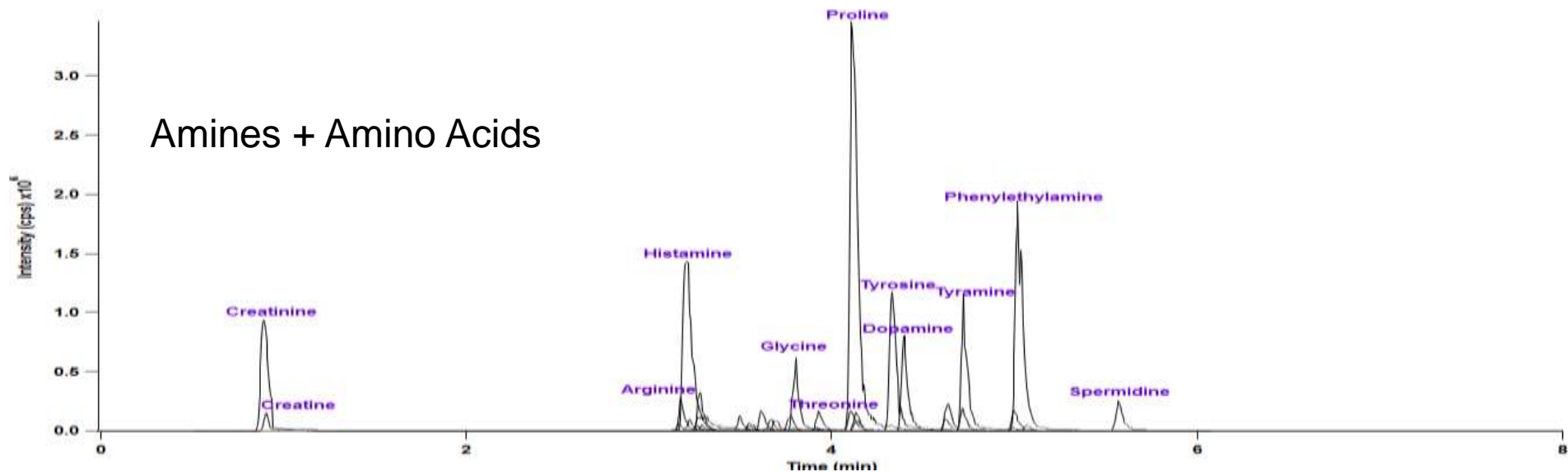
# Sample Preparation (LC-MS)



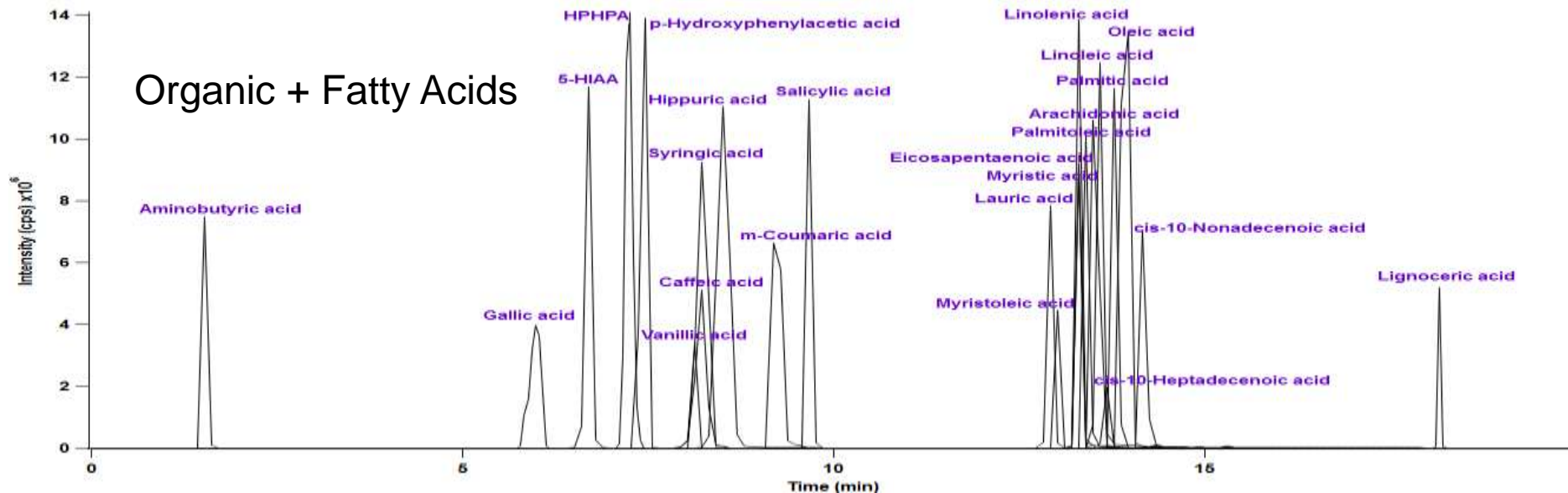


# Example LC Separations

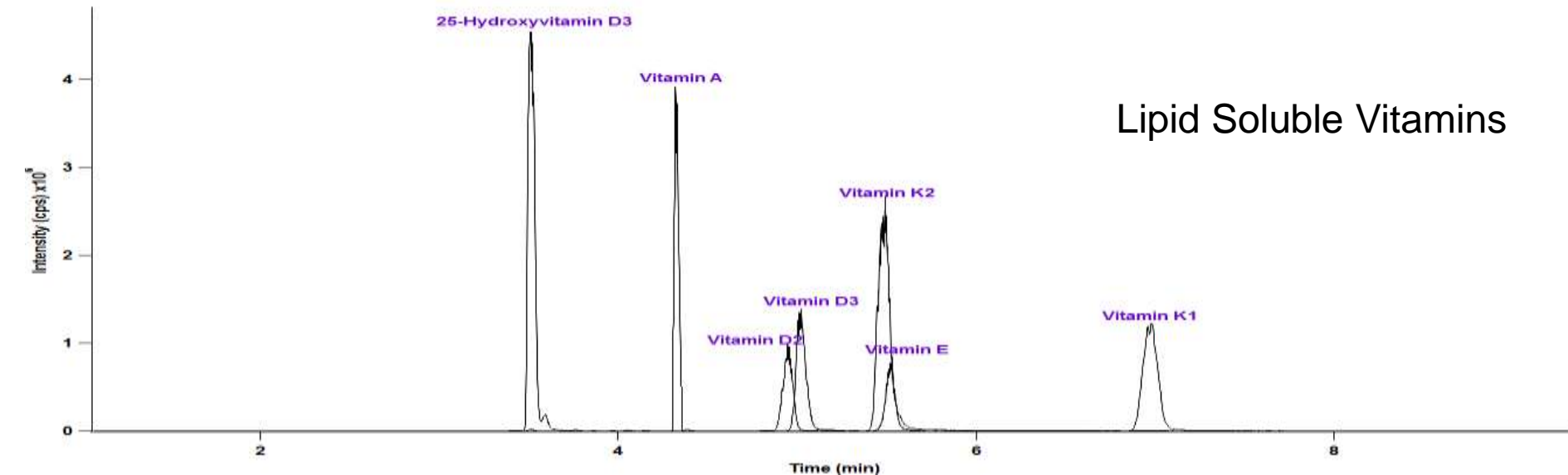
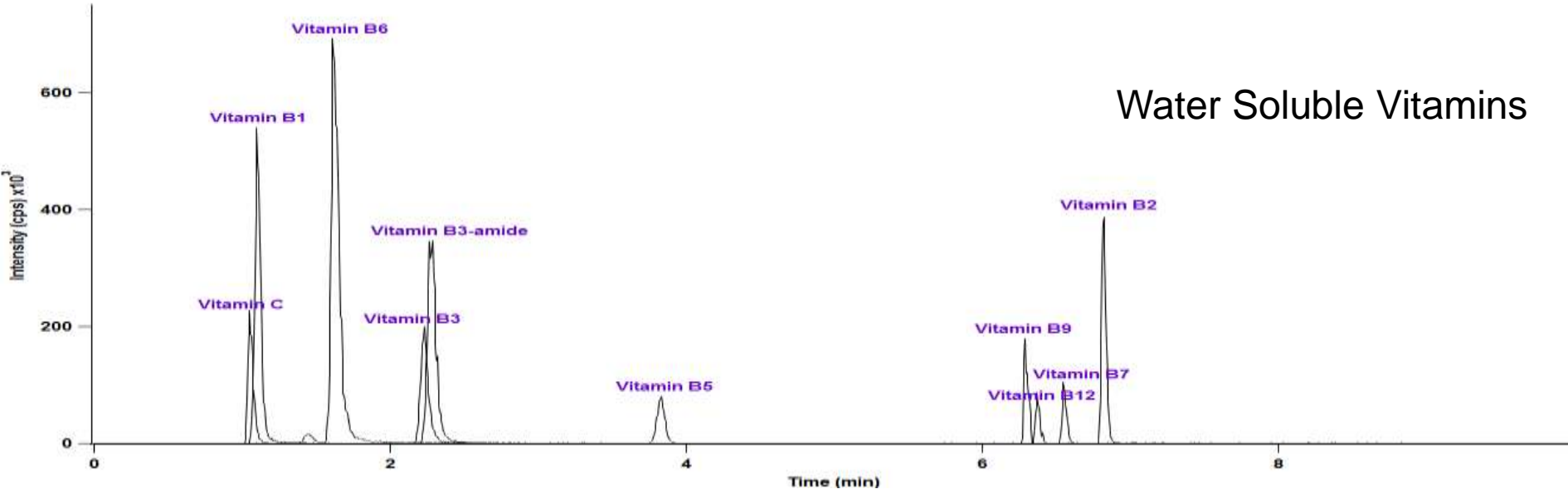
Amines + Amino Acids



Organic + Fatty Acids



# Example LC Separations



# Status & Outlook

- Validation of exposome/diet biomarker assay to be finished by Sept. 15, 2017
- “Official” release of ver. 1.0 exposome/diet biomarker assay by TMIC on Sept. 30
- Release of boxed kit version (for LC-MS only) for Sciex instruments on Oct. 30
- Release of boxed kit version for other triple quads for December (looking for collaborators)
- Version 2.0 to be released in 2018

# Conclusions

- **Metabolomics has played a key role in the first applications of precision medicine and precision nutrition (IEM diagnosis and monitoring)**
- **Metabolomics is more ubiquitous in the clinic than most people realize**
- **Metabolomics can play a key role in precision diagnosis, precision monitoring and developing precise diet/lifestyle “action plans”**
- **Metabolomics kits can reduce costs and enable precision medicine applications – including precision nutrition**

# Acknowledgements

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- Beomsoo Han
- Robert Fraser
- Pieter Cullis
- Craig Knox
- Mike Wilson
- Ana Marcu
- An Chi Guo



**Genome**Alberta



Western Economic Diversification Canada  
Diversification de l'économie de l'Ouest Canada



# Compound Classes in Kit #2

Classes	Food Sources
Vitamins	Meat, Fish, Milk, Fruits, Eggs, Vegetables, Beans, Pulses
Amino Acids & Derivatives	Meat, Poultry, Seafood, Eggs , Dairy, Beans & Nuts
Miscellaneous (Amines, Alkaloids)	Meat, Fish, Coffee, Tea
Organic Acids	Green Vegetables, Fruits, Dairy
Fatty Acids	Fish, Fish Oil, Seafood, Flaxseed, Walnut
Metals	Na, K, Ca, Zn - Food Consumption Pb, Cd, As , Al, Sr – Toxic Exposure