

# Precision Medicine – The Analytical Pipeline Behind Patient Remote Blood Sampling

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**Cedars Sinai**

Precision Biomarker Laboratories

[cedars-sinai.org/pbl](https://cedars-sinai.org/pbl)

# Challenges in Precision Medicine



Growing need for new approaches to precision health



Breadth and accuracy of biochemical assays matter



Telehealth coupled with off-site self-sampling could be revolutionary



# Paradigm Shift Towards Personalized Medicine

## Past & Today

We have a drug



Find me patients



## Today & Tomorrow

We have a patient



Find me treatment

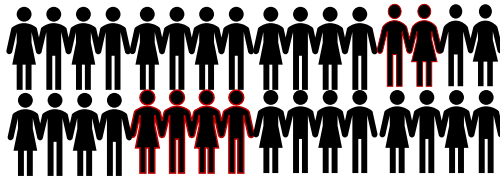
# Shift Towards a New Diagnostic Paradigm

## Past & Today

We have a drug



Find me patients



Subclassification

## Today & Tomorrow

We have a patient



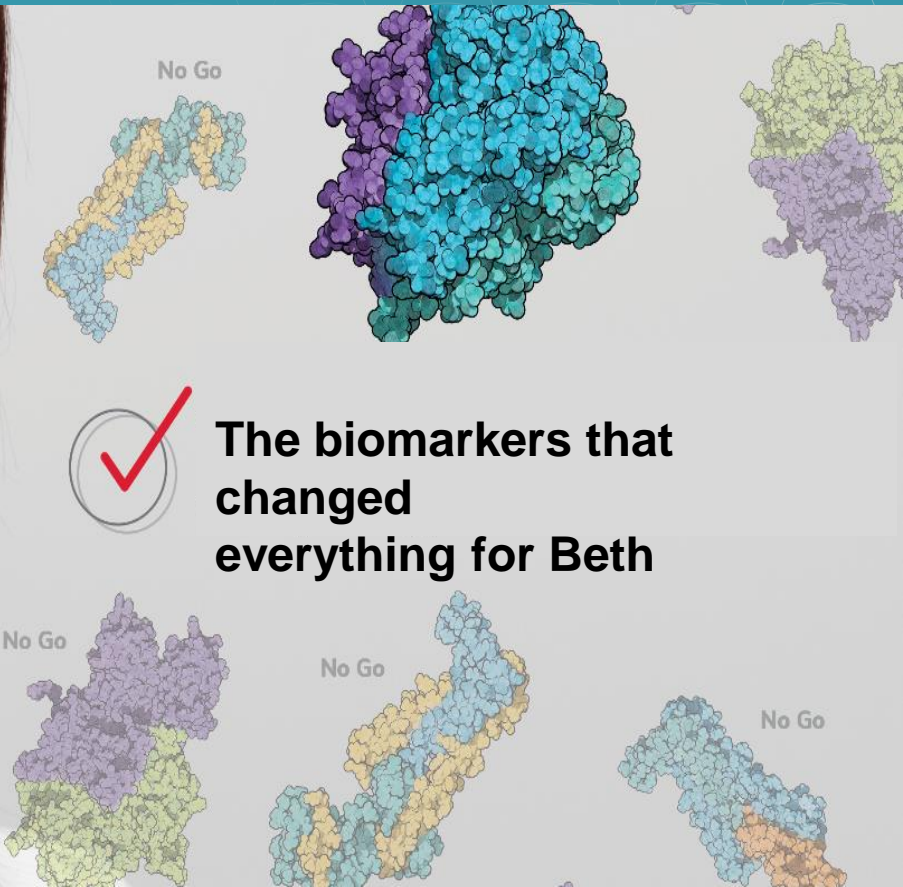
Find me treatment



Individual



# What is required to make this possible?

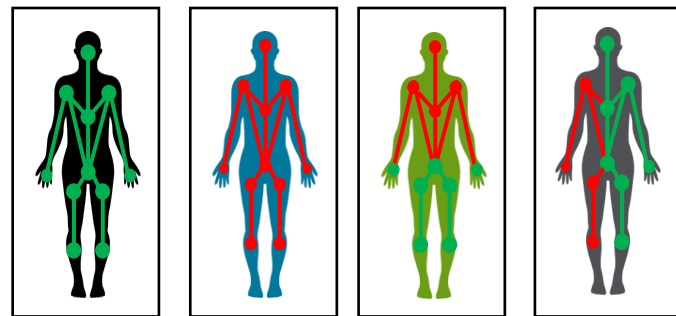
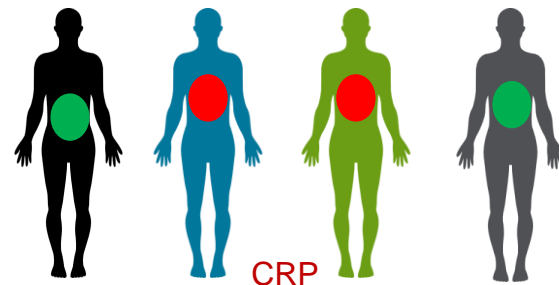


**The biomarkers that changed everything for Beth**

- From one disease to holistic approach
- From one marker to mechanistic network
- From one timepoint to a longitude tracking
- Telehealth and next generation of medical practice

# From one marker to mechanistic network

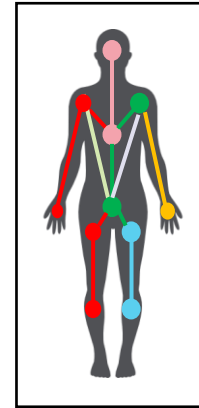
- Disease may not be represented by a single sentinel protein, but rather by a network of interconnected proteins
- At each disease stage, there can be multiple underlying processes
- Differential combinations of networks should better define an individual
- Protein network dynamics allow differentiation between people, disease states and time
- There is a need to deconvolution of multiple networks in order to match the multiple biological processes



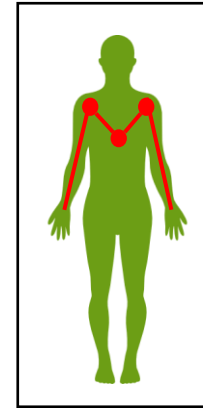
Acute Phase Protein (APP) and Health Surveillance (HS)

# From one marker to mechanistic network

- Targeted Discovery assay provides quantification across broad swath of biology and is disease-dependent. This is DIA-MS assay.
- Health Surveillance (HS) assay is comprised of 52 proteins representing 9 pathological processes including innate immunity, vascular response, platelet activation, and lung, heart and kidney function. This is a MRM assay.
- Acute phase protein (APP) assay is comprised of 11 proteins representing acute phase response. This is an immuno-MRM assay.



HS assay



APP assay



Targeted discovery

Pathway specific

Single protein represents pathway

**Holistic**  
**DIA-MS**



monitoring time  
↔

**Disease-specific**  
**APP-iMRM**

**Selective**  
**CRP**



IDENTIFIED CLINICAL NEED  
& BIOMARKER SOLUTION

PROTEOMICS BIOMARKER  
TARGETED DISCOVERY

TECHNICAL  
VIABILITY

TRANSLATIONAL  
DEVELOPMENT

CLINICAL TRIALS

CLINICAL USE

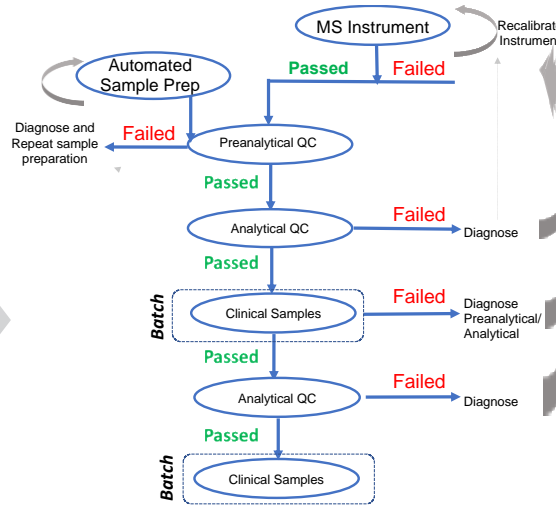
## Targeted discovery



Holistic

DIA-MS

1. Reproducibility
2. Precision
3. Linearity



Digestion block layout

	1	2	3	4	5	6	7	8	9
A	BK	4	12	20	27	35	43	51	59
B	H-QC	5	13	21	28	36	44	52	60
C	M-QC	6	14	22	29	37	45	53	61
D	L-QC	7	15	M-QC	30	38	46	54	62
E	1	8	16	23	31	39	47	55	63
F	2	9	17	24	32	40	48	56	64
G	3	10	18	25	33	41	49	57	BK
H	BK	11	19	26	34	42	50	58	M-QC

### Instrument QC

- Hela Cell lysate Digest spike with synthetic peptides – **DIA**
- Beta-gal Ecoli Digest spiked with isotopically labeled standards – **MRM**
- 8 point calibration curve – **MRM**
- Internal Retention time standards – **DIA**

### Pre Analytical QC: MRM & DIA

- H-QC-neat Golden West human Plasma
- M-QC-1:2 dilution of H-QC with chicken plasma
- L-QC -1.5 dilution of H-QC with chicken plasma

### Analytical QC: MRM & DIA

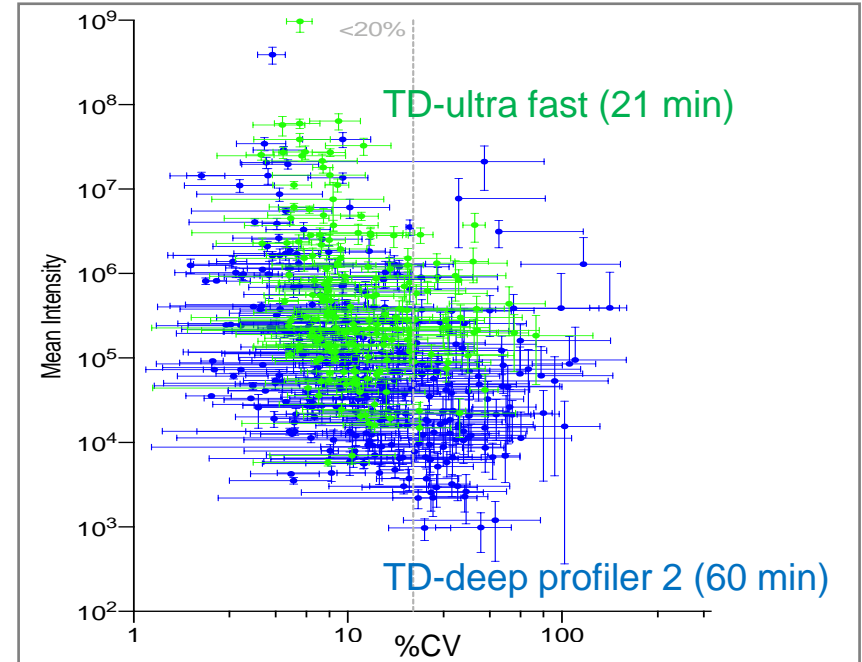
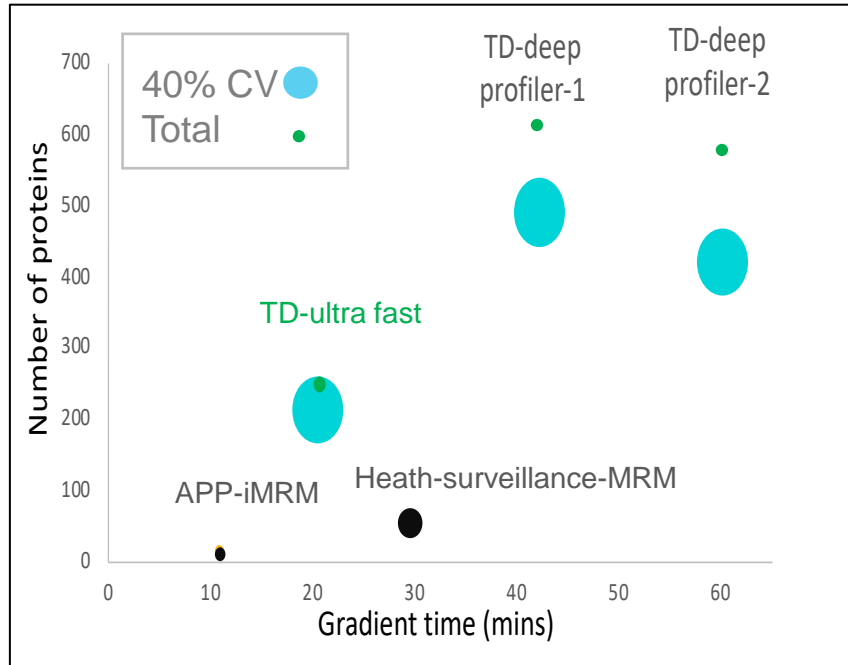
- Pre Digested H-QC- and spiked with internal standards

### Sample QC

- Exogenous Beta-Gal protein – **DIA & MRM**
- Synthetic isotopically peptides representative of assay targets – **MRM**
- Internal Retention time standards – **DIA**

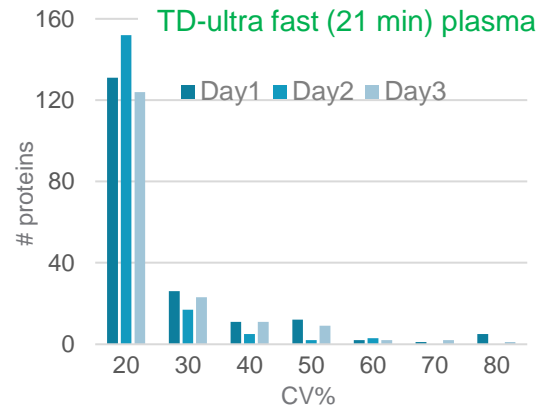
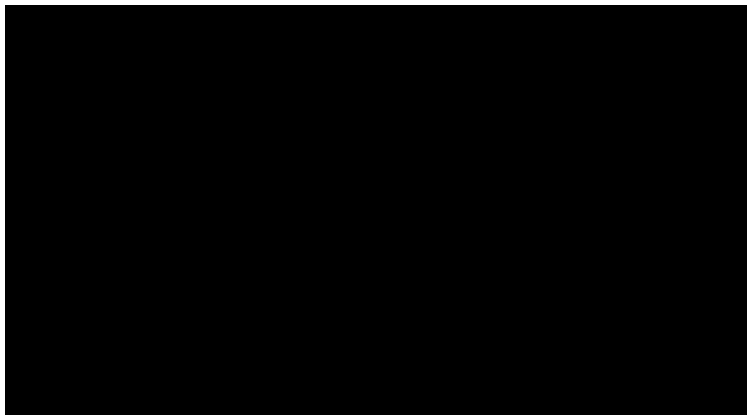
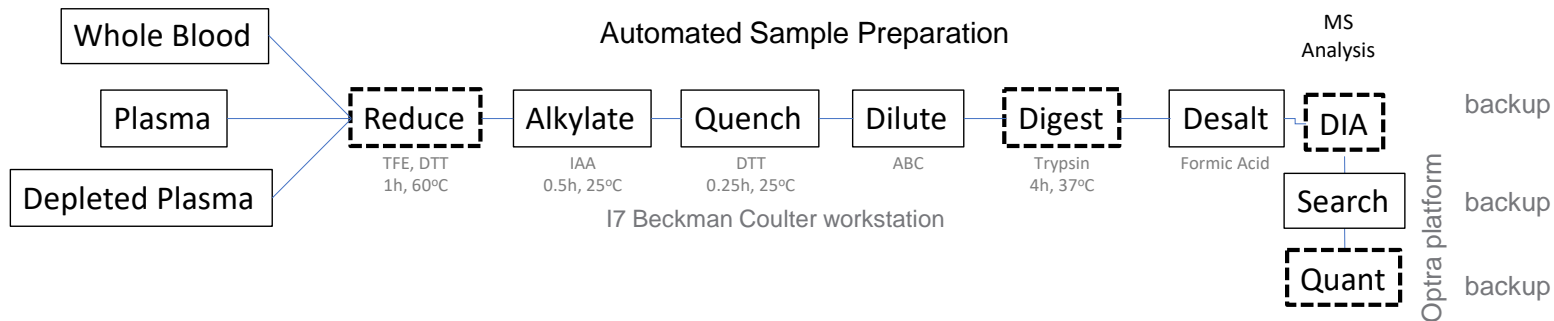
10% QC samples and 10% redo rate

# Leveraging mass spectrometry for reproducible and accurate mechanistic assays



Based on 3x3x3 reproducibility study

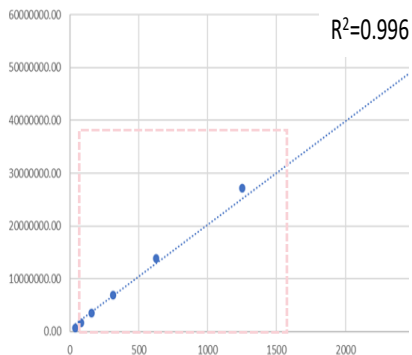
# Modular pipeline maximizes automation of sample preparation, data analysis and reporting



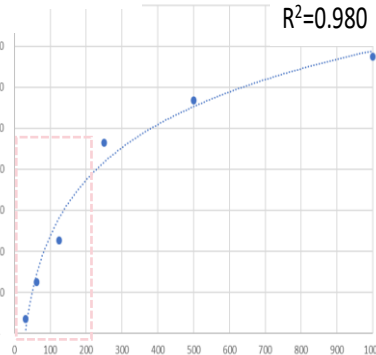
# Breath and Depth of Proteome Coverage vs. throughput (60 vs 18 samples/day)

TD-deep profiler 2 (60 min)

TD-ultra fast (21 min)



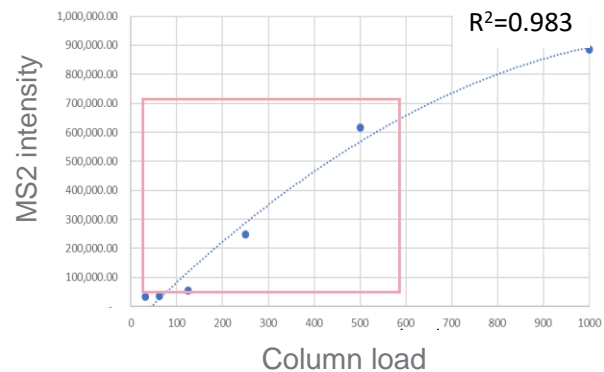
Column load



## Linear and Reproducible Analysis of Naive Plasmas

Enriched Biological Pathways	# quantifiable proteins (<40% CV)	
	Native_21M	Native_60M
Neutrophil degranulation	21	134
Leukocyte mediated immunity	49	98
Neutrophil mediated immunity	28	74
Complement and coagulation cascades	62	73
Humoral immune response	38	54
Platelet activation, signaling and aggregation	29	42
Acute inflammatory response	19	31
Lipid Metabolism		25
Signaling by Interleukins		26
Triglyceride metabolic process	6	
Adaptive immune response		38
Innate immune response		76
Lipid catabolic process		13
Response to reactive oxygen species		17
Angiogenesis		31
VEGFA-VEGFR2 Signaling Pathway		19
Platelet degranulation		9
Interleukin-4 and Interleukin-13 signaling		12
Blood vessel endothelial cell migration		8

# Effective increase in breadth and depth by combining two workflows



5x3x3 using 21 min gradient quantified 581 proteins (82%) amongst all replicates across all days

- Depleted plasma preparation (camel Ab top 14 plasma proteins/96 well format) is linear and reproducible.
- Depleted plasma combined with naïve plasma using 21 min gradient provides broadest coverage for less time (TD-deep profiler 1).

Enriched Biological Pathways	# quantifiable proteins (<40% CV)		
	Naïve_21M.	Naïve_21M. +Depleted_21M	Naïve_60M
Neutrophil degranulation	21	43	134
Leukocyte mediated immunity	49	79	98
Neutrophil mediated immunity	28	53	74
Complement and coagulation cascades	62	84	73
Humoral immune response	38	55	54
Platelet activation, signaling and aggregation	29	40	42
Acute inflammatory response	19	24	31
Lipid Metabolism		23	25
Signaling by Interleukins		20	26
Triglyceride metabolic process	6	7	
Adaptive immune response		39	38
Innate immune response		72	76
Lipid catabolic process		4	13
Response to reactive oxygen species		17	17
Regulation of interleukin-6 production		11	-
Angiogenesis		26	31
VEGFA-VEGFR2 Signaling Pathway		13	19
Platelet degranulation		6	9
Antigen cross-processing and presentation		12	
Proteasome Degradation		8	
Interleukin-1 signaling		5	
Cellular response to hypoxia		5	
Triglyceride biosynthetic process		7	
Interleukin-4 and Interleukin-13 signaling			12
Blood vessel endothelial cell migration			8

# Center for Undiagnosed Patients (CUP): meets a critical unmet clinical need and provides a unique opportunity to understand the complexity of individual disease diversity.

Programs & Services Center for the Undiagnosed Patient Share Email Print

## Center for the Undiagnosed Patient

Sometimes, in spite of efforts by multiple doctors, a patient with a chronic condition is unable to get a diagnosis. Undiagnosed patients often have rare disorders. At Cedars-Sinai, we are dedicated to finding ways to diagnose those rare conditions.

Expert Team

Adult Center

Pediatric Center



**Adult Center**

Cardiologists, geneticists, neurologists, endocrinologists, gastroenterologists and infectious disease specialists are all part of the team helping to diagnose a patient's condition so that the patient can achieve optimal health and wellness.



**Pediatric Center**

Complicated evaluations sometimes require a "kid-friendly" environment. The Pediatric Center aims to provide answers and insights to families with children affected by undiagnosed disorders.

<https://www.cedars-sinai.org/programs/undiagnosed-patient-center.html>

The notion of “diagnosis” is open to a re-think and is dependent on a holistic approach.

### Potential clinical outcomes

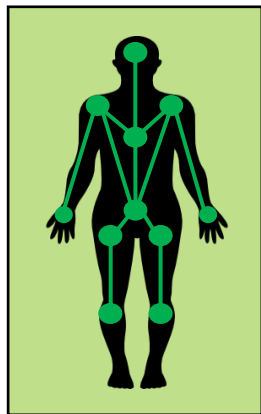
- Diagnosis of new rare disease
- New syndromes
- Unusual presentation of common diseases
- Multiple overlapping diseases
- Rule out of disease

# What is the benefits for clinical release of a subset of proteins from a DIA-MS.

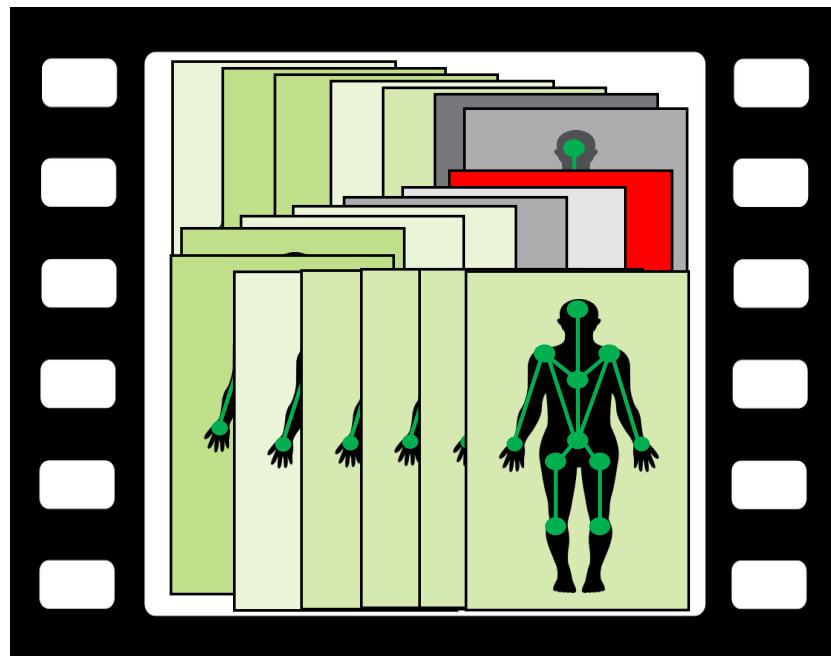
Similar to CLIA laboratories carryout whole genome sequencing with release of subset of gene as the clinical panel, can we create sufficient large panels that can be efficiently run with the accuracy that allows release of subset of clinical data?



# Dynamic disease networks continuously evolve over time



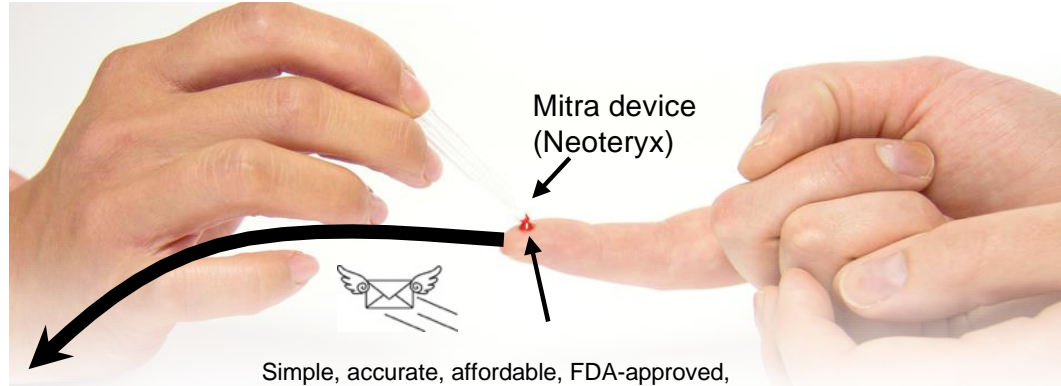
Dynamic Network



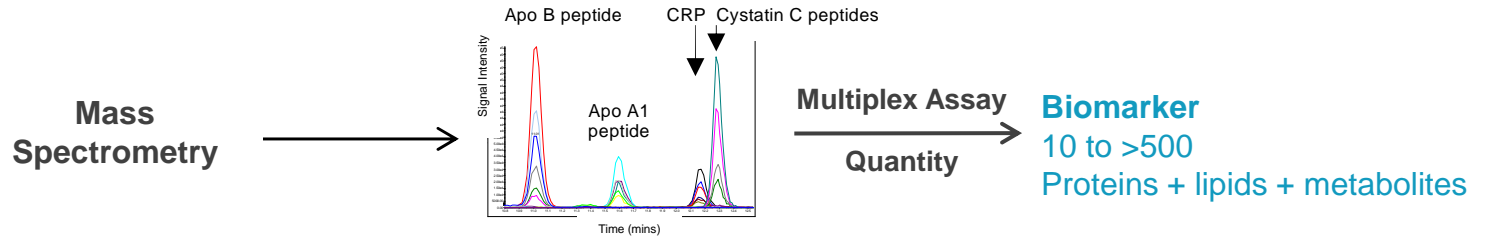
Dynamic Development



# Transformative technology: 10ul blood draw, any time, any place, by anyone



Simple, accurate, affordable, FDA-approved, automatable microsampling



# Transformative technology

10 ul blood draw, any time, any place by any one.

- Instructions
- Devices in claim shell
- Bandage
- Lances
- Foil bag with desiccant
- Addressed envelope



Tasso



HemaSpot

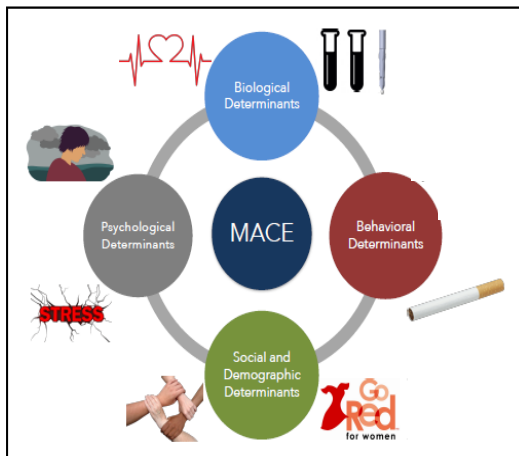


Classic filter paper

# CIAPM – California Initiative to Advanced of Precision Medicine

## Predict MACE

- Heart attacks
- Sudden death due to CVD
- Arrythmia
- Re-hospitalization due to heart failure



200  
patients

## **Biomarkers**

- C-reactive protein (CRP)
- Brain Natriuretic peptide (BNP)
- Cardiac Troponin I (cTnI)
- Apolipoprotein Panel -Mitra tips\*

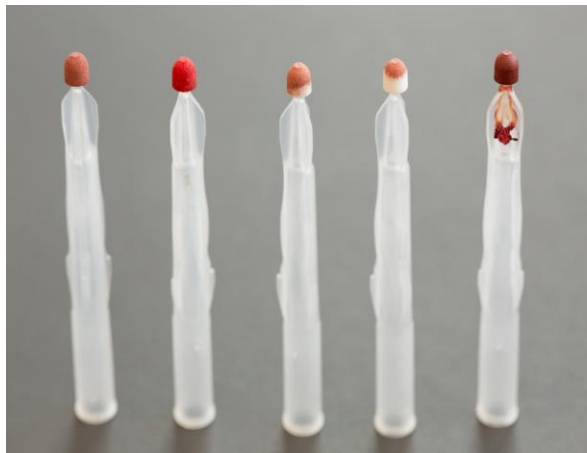
## **Patient Reported Outcomes and Informatics (PRO & PRI)**

- Fitbit
- Exit Questionnaires

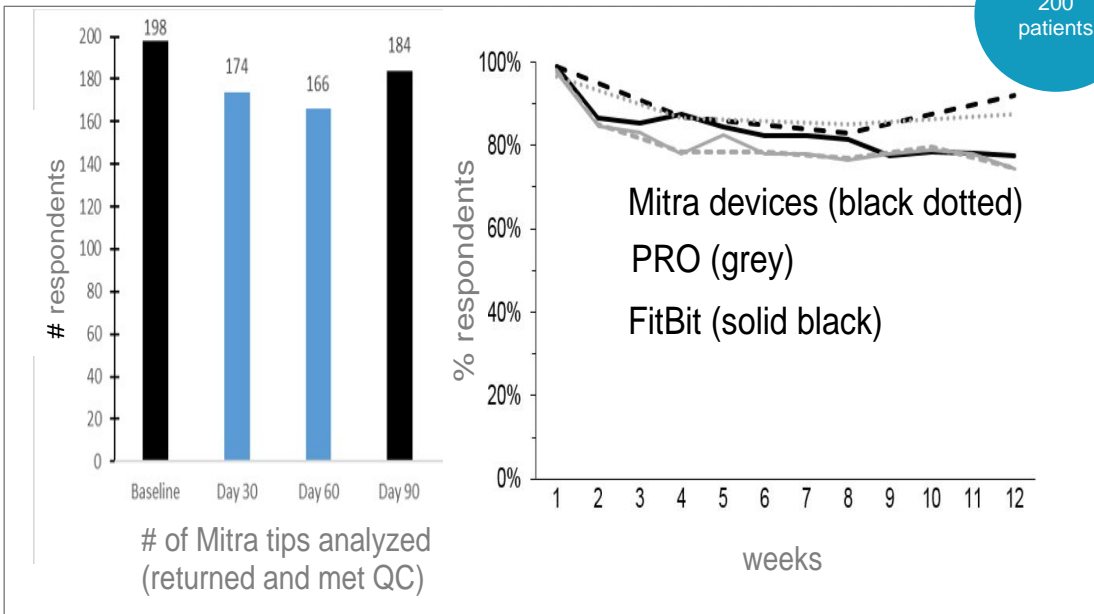
## **Case/Control Group**

- Lipids - (SCIEX Lipidyzer)
- Deep discovery - (ThermoFisher Scientific)

# Optimal performance: Requires Standard Operating Procedure and Quality Control



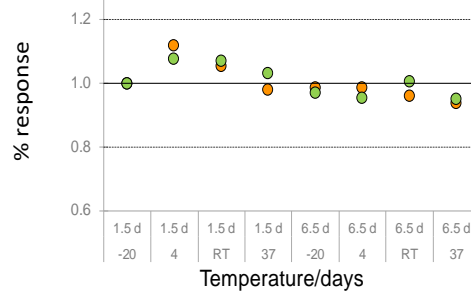
✓ ✓ X X X



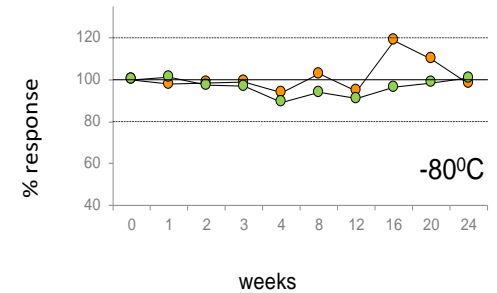
# Optimal performance: Requires Standard Operating Procedure and Quality Control

- Device and sample performance
- Patient compliance
- Assay performance
- Assay relevance

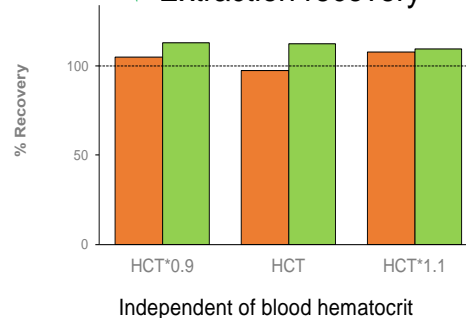
✓ Short-term stability of Mitra Tips



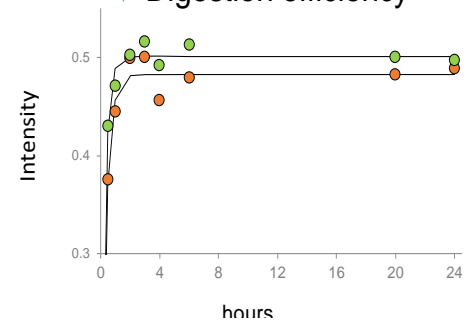
✓ Long-term stability of Mitra Tips



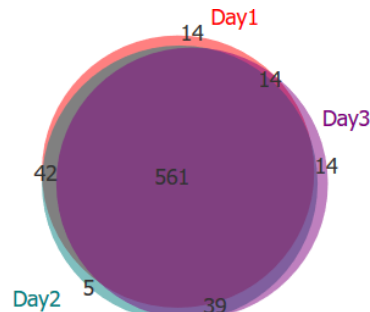
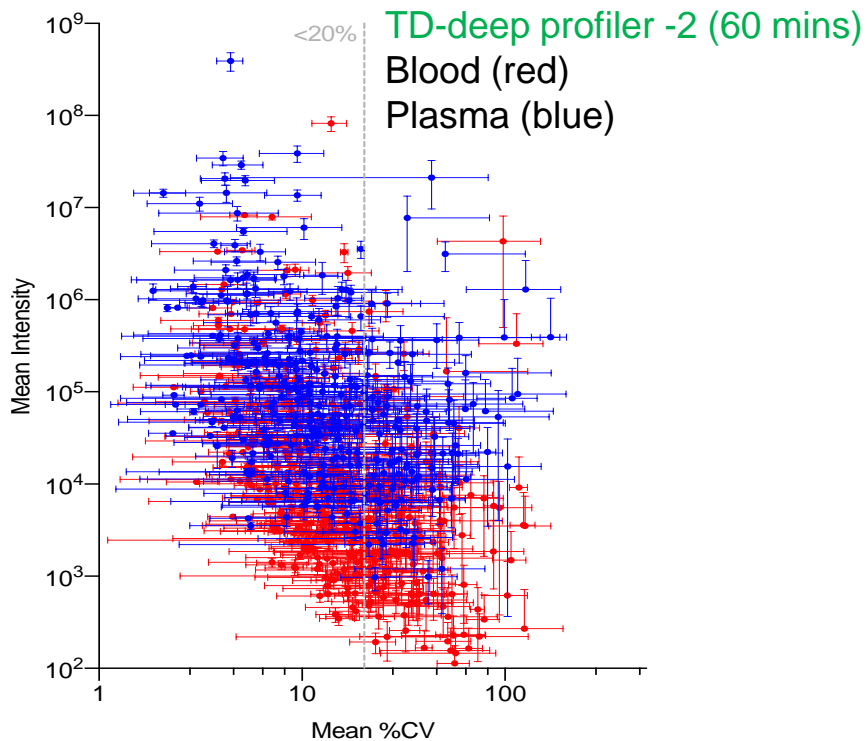
✓ Extraction recovery



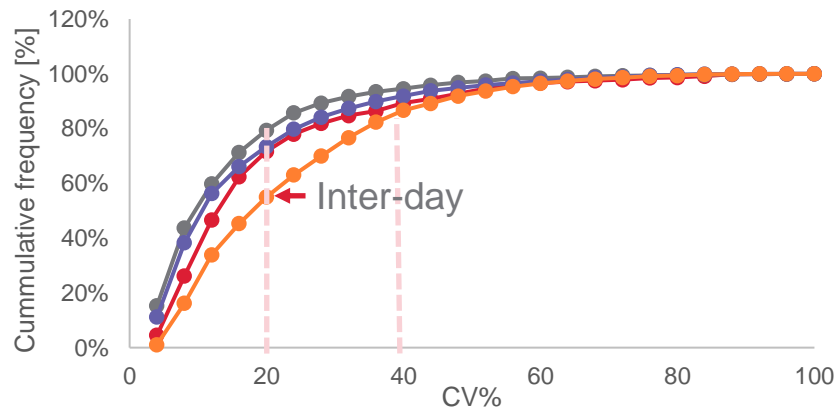
✓ Digestion efficiency



# Blood is a very interesting biomatrix



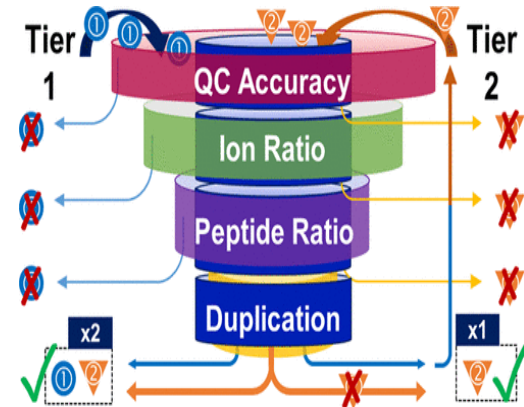
Healthy	# proteins (5x3x3 study)	
	ExoSep-Exploresis	Phenomix-Exploresis
20%CV	196	413
40%CV	279	559
Total	353	679



# Another challenge is the need for automation of peptide quantification.

## 199 mid-risk cardiovascular patient monthly (4 time points) van den Broek *et al*, JPR in press

- 11 Apolipoprotein multiplex panel LC-SRM-MS
  - Quantifier and qualifier peptides/protein
  - endogenous and N<sup>15</sup> internal standards (isotopologues)
- 199 individual with 4 timepoints
- 741 timepoint samples as single or duplicate = total of 1127 samples
- 11 proteins @ 2 peptides @ 3 transitions @ 2 isotopologues = 72574 chromatographic peaks



	albu	apoA1	apoA2	apoA4	ApoB	ApoC1	ApoC2	ApoC3	ApoE	Clus	HBA
Tier 1	95	84	87.8	95	86.7	78	89.1	61.5	85.1	96	74
Excluded	5	11	10	4	6	9	8	24	6	2	7
Rescued	0.2	5	2.6	1	7.3	13	2.9	14.5	8.9	2	19

# Real world example: undiagnosed patients

Center for the Undiagnosed Patient



## Center for the Undiagnosed Patient

Sometimes, in spite of efforts by multiple doctors, a patient with a chronic condition is unable to get a diagnosis. Undiagnosed patients often have rare disorders. At Cedars-Sinai, we are dedicated to finding ways to diagnose those rare conditions.



### Adult Center ▶

Cardiologists, geneticists, neurologists, endocrinologists, gastroenterologists and infectious disease specialists are all part of the team helping to diagnose a patient's condition so that the patient can achieve optimal health and wellness.



### Pediatric Center ▶

Complicated evaluations sometimes require a "kid-friendly" environment. The Pediatric Center aims to provide answers and insights to families with children affected by undiagnosed disorders.

CUP meets a critical unmet clinical need and provides a unique opportunity to understand the complexity of individual disease diversity.

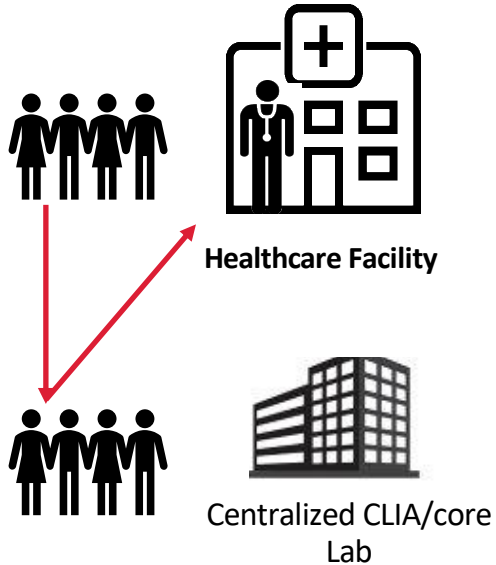
- New syndromes
- Rare genetic diseases
- Complex diseases
- Known disease with rare clinical presentation

**The notion of “diagnosis” is open to a re-think.**

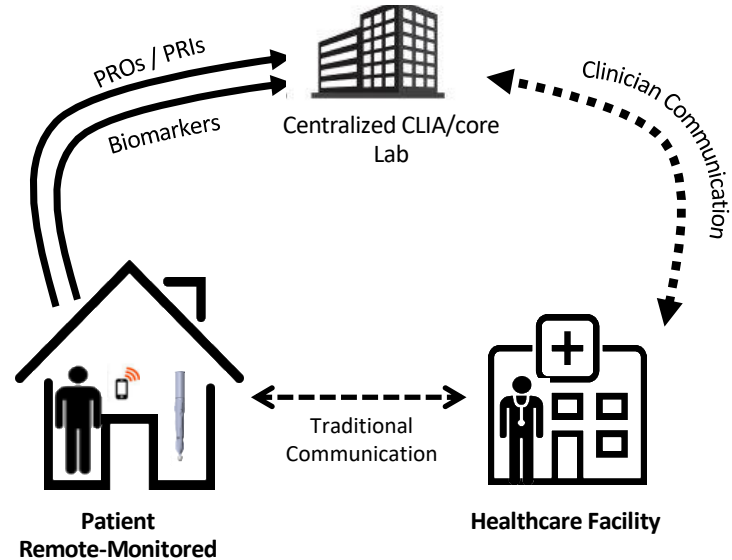


# Is there added value to patient sampling in context to telemedicine?

## Past & Today



## Today & Tomorrow



# Real world example: Covid-19 and the Corale Studies

Hypothesis: Even Covid-19 positive individuals who were asymptomatic or who had moderate clinical presentations and did not require hospitalization, may develop multiorgan dysfunction over time.



## *Coronavirus Risk Associations and Longitudinal Evaluation Study*

*corale* /ko'rale/ (Italian)

*literal translation: choral*

*figurative translation: chorus, unanimous*

*On March 14, 2020, quarantined Italian citizens organized to sing from their balconies to create community during this era of coronavirus.*

*We were inspired.*

# Real world example: Covid-19 and frontline healthcare workers



## Longitudinal Frontline Nurses study:

Mega-kit for at home sampling for 6 weeks

- Questionnaire
- Microbiome screening
- Neoteryx Mitra self-blood microsampling
  - seroconversion
  - metabolomics
  - acute phase protein markers
  - discovery proteomics
- Tasso self-blood/plasma microsampling
  - Seroconversion (Abbott clinical assay)

# Real world example: healthcare worker cohort >6000 employees/3 weeks with a 4.8% anti-covid IgG positivity.



IRB No: [IRBNo]  
Approval Date: [ApproveDate]  
Expiration Date: [ExpireDate]

*The Coronavirus Risk Associations and Longitudinal Evaluation (CORALE) Study*

## BASE Healthcare Workers Sub-Study

### HEALTHCARE WORKERS NEEDED FOR RESEARCH

We are working to understand how antibody testing could inform our ability to keep healthcare workers safe and healthy in the era of COVID-19. In addition to studying how well antibody testing works, we are also looking at how measures of antibodies and other immunity biomarkers are related to exposures and health outcomes across all areas and at all levels of our workforce.

### ELIGIBILITY CRITERIA

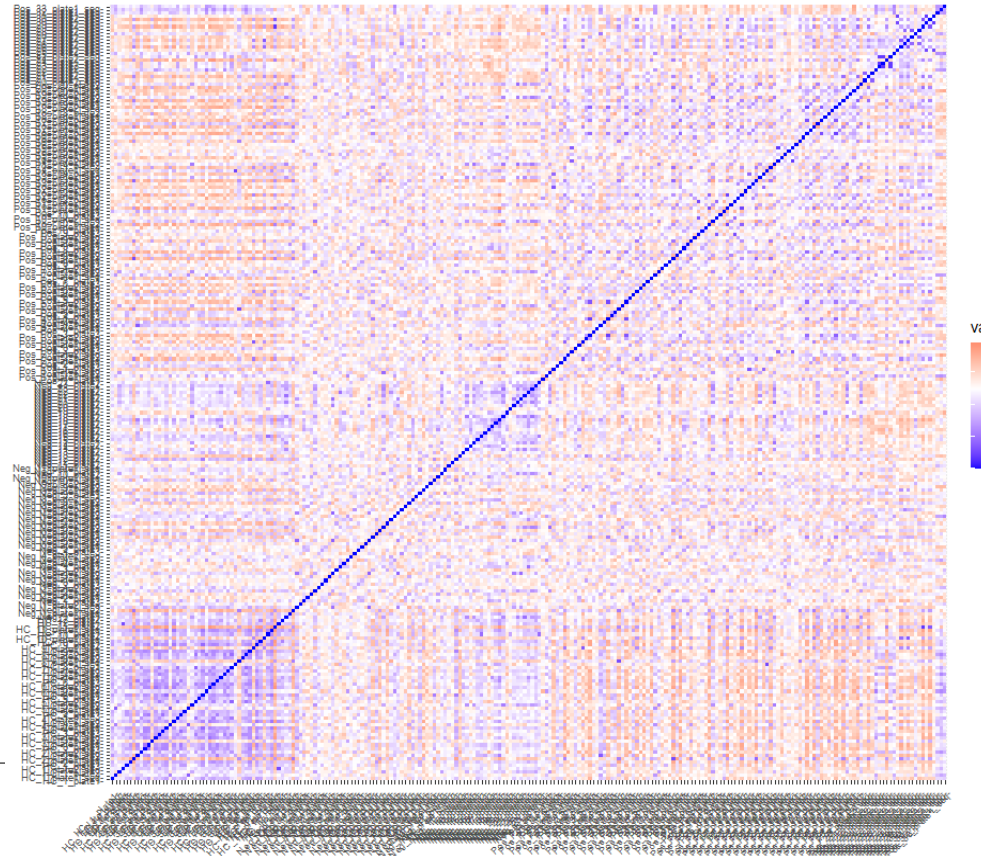
To participate, you must be 18 years or older and actively employed as a healthcare worker at a facility that is caring for patients with COVID-19.

### WHAT'S INVOLVED

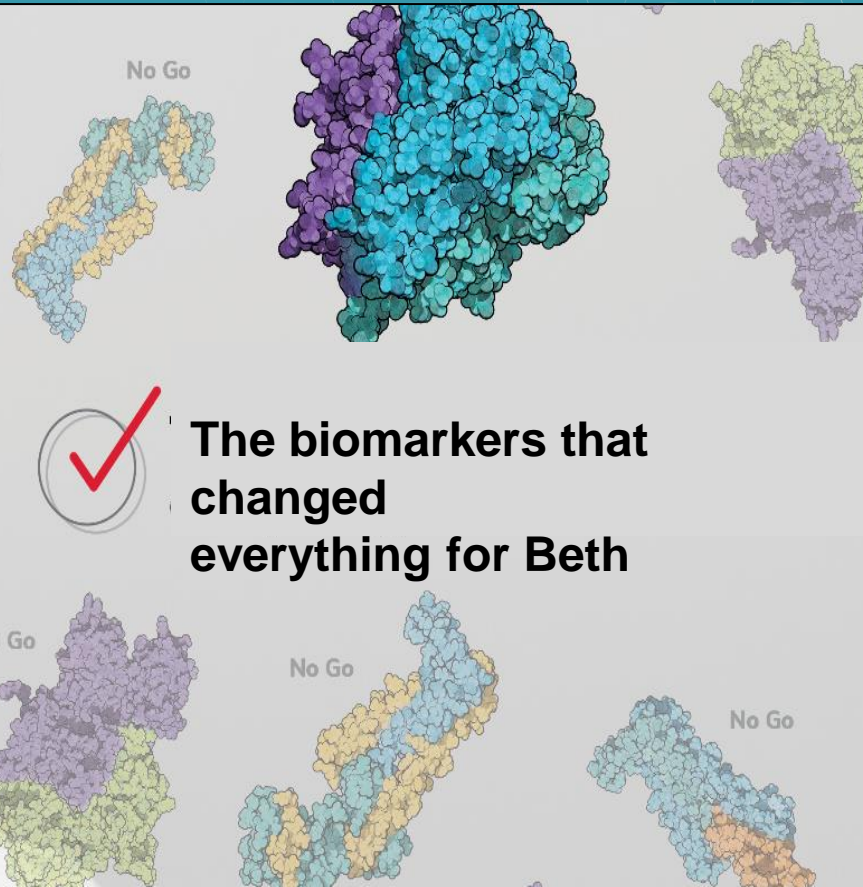
- A questionnaire administered by web, mail, or phone.
- A blood collection drawn at or near your work site.

### CONTACT US

To learn more about the study, you can visit us at [corale-study.org](http://corale-study.org).  
If you are interested in participating, you can contact us at [humanphysiology@cshs.org](mailto:humanphysiology@cshs.org).

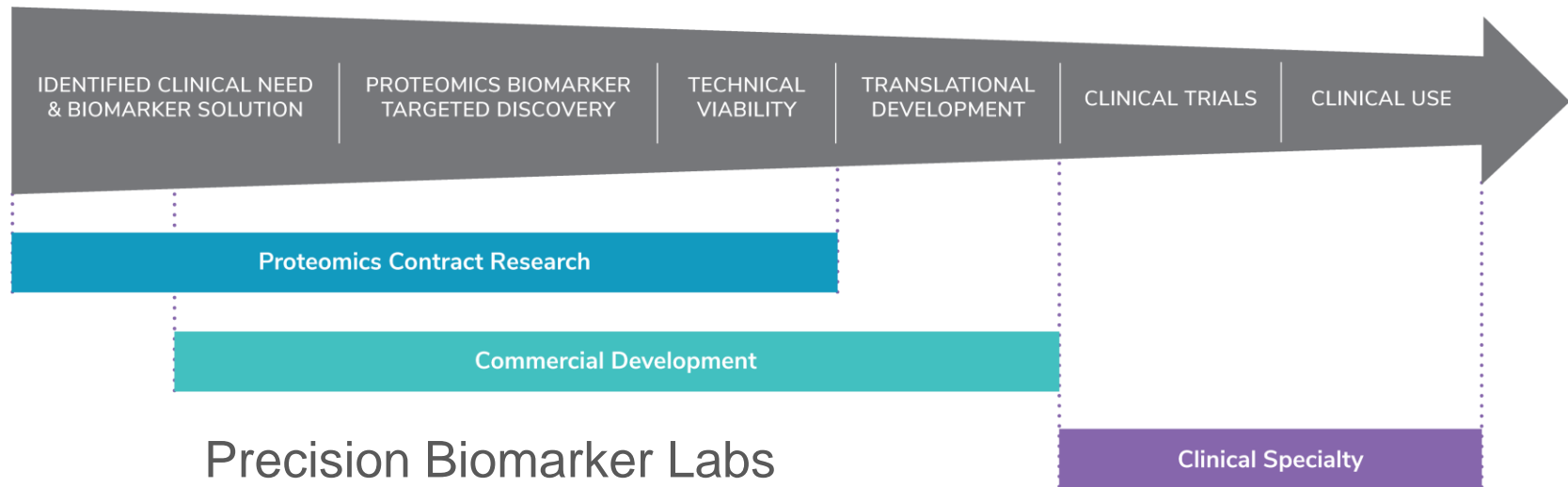


# What is possible?



- From one disease to holistic approach
- From one marker to mechanistic network
- From one timepoint to a longitude tracking
- Telehealth and next generation of medical practice

# Meeting your needs: From biomarker discovery to commercialization



Precision Biomarker Labs  
3 labs under one umbrella

# PBL customize individual services or integrated solutions



## Proteomics Contract Research

- Discovery and early research support
- Ready-to-go, targeted assays
- Biomarkers of biological and clinical relevance
- Leading mass spectrometry (MS) technology and expertise



## Commercial Development

- Analytical methods for clinical and commercial deployment
- Access to clinical expertise and cohorts
- Bioinformatic and data analytic framework for clinical investigation



## Clinical Specialty

- Completes translation to clinical application
- Develops assays with defined clinical validity
- Supports validated assays from external sources (e.g. niche products, support for clinical result interpretation)

# The Duality of Precision Medicine



Growing need for new approaches to precision health



Breath and accuracy of biochemical assays matter



Telehealth coupled with off-site self-sampling could be revolutionary



Perturbations to determine Personal proteomic response



Rapid screen to determine personalized drug response



Expedite therapeutic decisions for each person





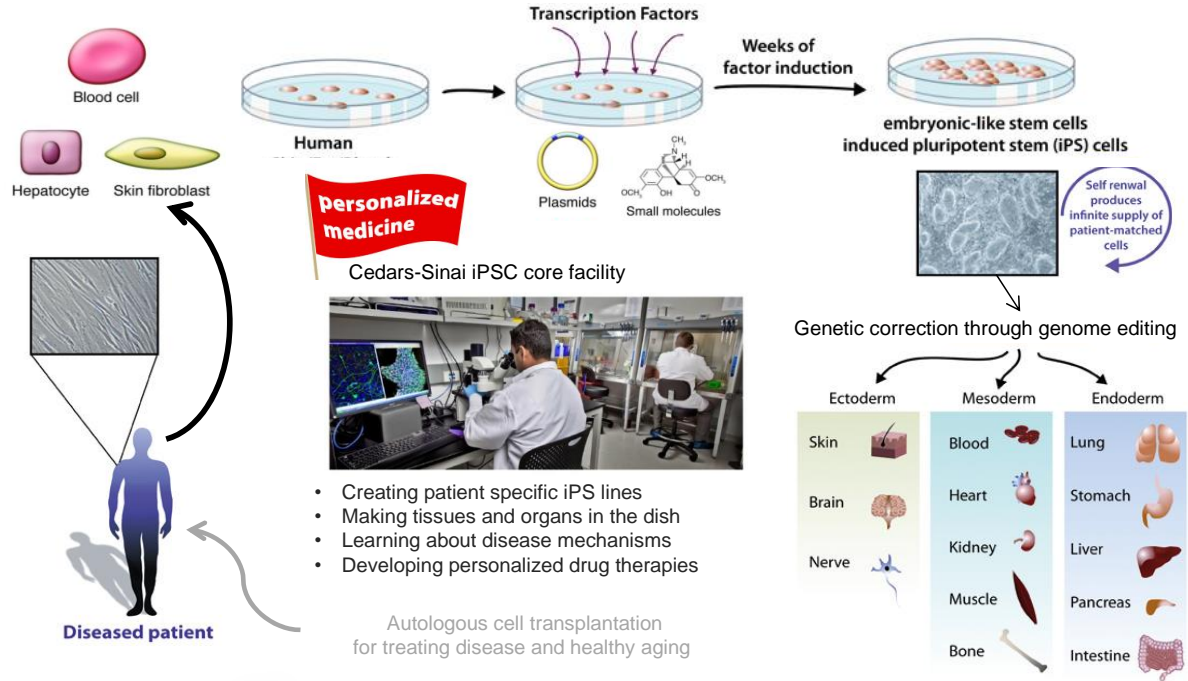
# Human induced pluripotent stem cells retain the genetic make up and can be derived into organoids and organs as models of disease

## Clinical Selection

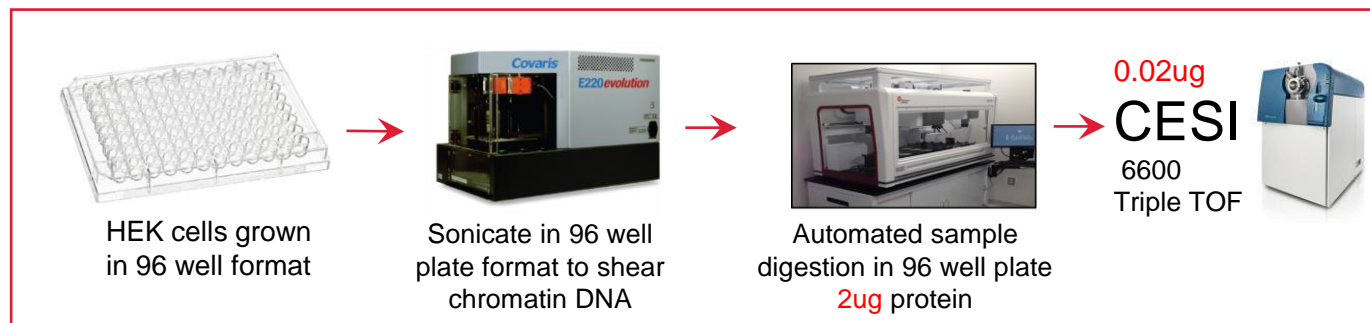
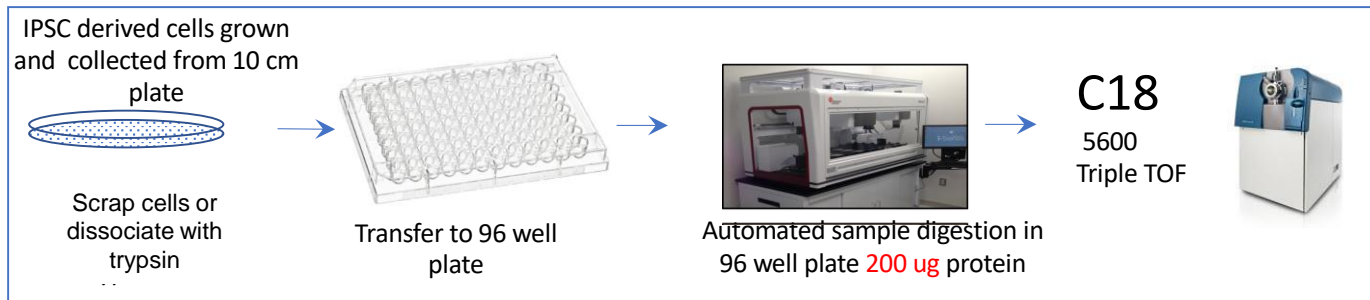
Drug selection based on your own iPSC derived organ as a patient surrogate

## Mechanistic Drug

Population wide perturbation for drug development to capture biological diversity.

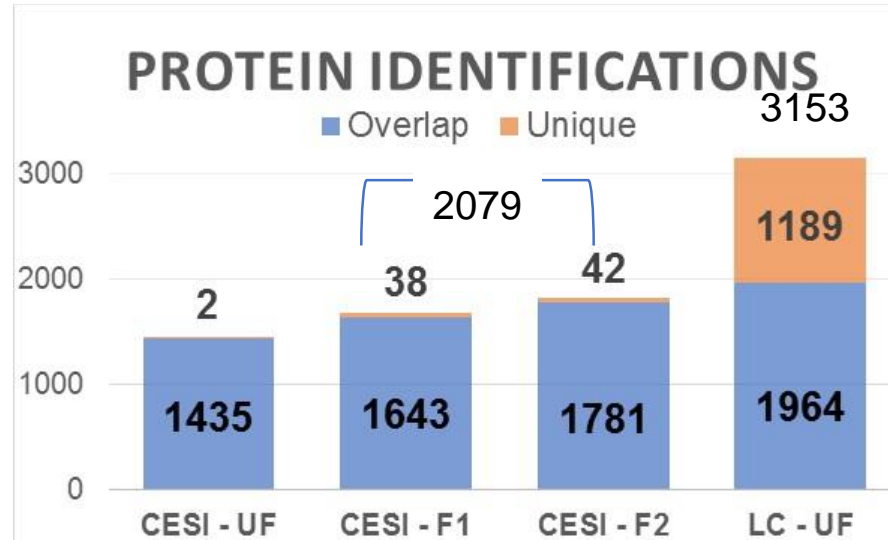


# Reproducibility and throughput increased by sample digestion automation of Answer ALS iPSC-derived motor neurons



Covaris: highly-controlled, isothermic and focused acoustic energy

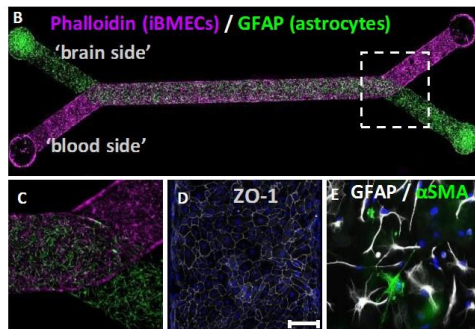
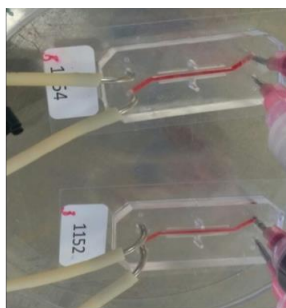
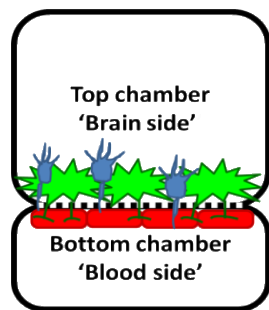
Two step hydrophobic fractionation increases proteome coverage with CESI and still requires ~ the same amount of MS run time.



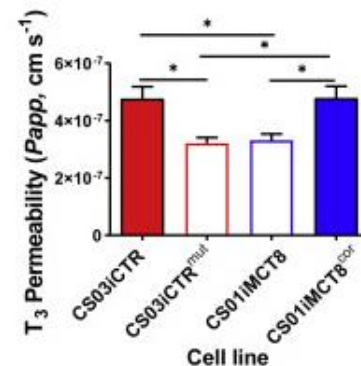
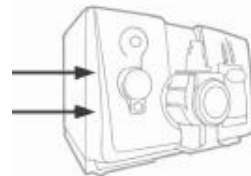
~ 66% of the proteome coverage by CESI (with 2 step fraction) compare to LC which has a longer gradient and used ~10 x quantity.

Andrea Matlock, Vineet Vaibhav, Farzin Gharahdaghi

# Patient derived iPSC organ-on a-chip can provide a model of the blood brain barrier



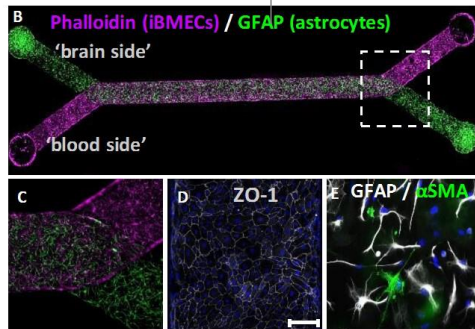
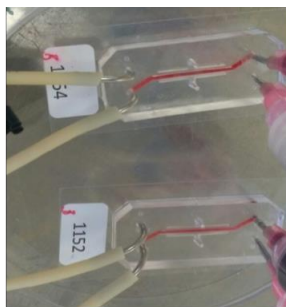
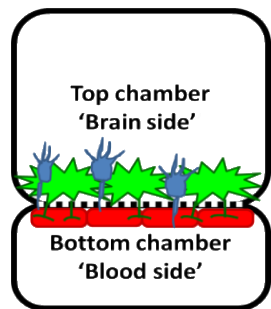
Clive Svendsen, Gad Vatine, Michael Workman, Westin Spivia, Dawn Chen



iPSC-derived endothelial cells make capillary which human blood is pumped through

Measurement of small molecule and proteins that cross the blood brain barrier.

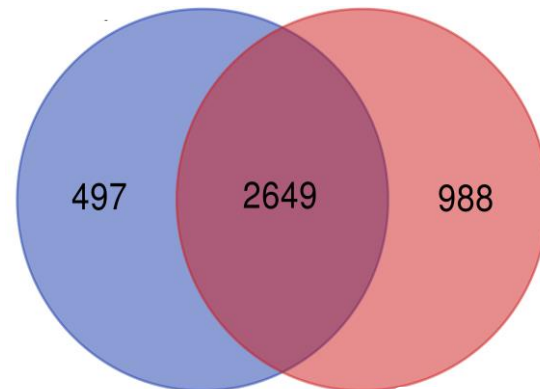
# Patient derived iPSC organ-on a-chip can provide a model of the blood brain barrier



Clive Svendsen, Gad Vatine, Michael Workman, Westin Spivia, Dawn Chen

IPSC-derived endothelial cells  
make capillary which human  
blood is pumped through

## Proteomic DIA-MS



CHIP  
n = 10

Plate  
n = 38



# The Next World of Precision Medicine

- Expanding the physician's toolbox via molecular proteotyping that links an individual's proteome and phenotype over time
- Defining the interconnections between an individual's proteotype and risk/disease status for the development and application of individualized diagnostics and therapeutics



# Those that matter the most

## Advanced Clinical Biosystems Research Institute (ACBRI)

### The Smidt Heart Institute (Van Evk lab)

Qin Fu (HIT director)  
**Angie Mc Ardle** (MS)  
Simion Kreiser (MS)  
Rahki Pandey (MS)  
**Aleksandra Binek**  
**Blandine Chazarin**  
Danica Manalo  
Alejandro Rivas  
Koen Raedschelders

Vidya Venkatraman (BI director)  
Niveda Sundararaman  
James Go  
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Aleksandr Stotland  
Chris Murray  
Kelly Moaupi  
Vineet Vaibhav  
Victoria Dardov

Aaron Robinson  
Rowann Mostafa  
Weston Spivia\*  
Vineet Vaibhav  
Shenyan Zhang\*  
Mitra Mastali\*  
Irina Chernysheva  
Casey Johnson\*  
Andrea Matlock\*  
Ron Holewinski\*

## Precision Biomarker Labs Cedars-Sinai Med. Center

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Anders Berg  
Annie Moradian  
Mitra Mastali  
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Casey Johnson  
Irene van den Broek\*

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## Neoteryx

**Fasha Mahjoor**  
**Stuart Kushon**  
**Kim Chansky**

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Joe Ebinger  
Koen Raedschelders  
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and many more

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Michael Kowlaski  
Christie Hunter

## Others

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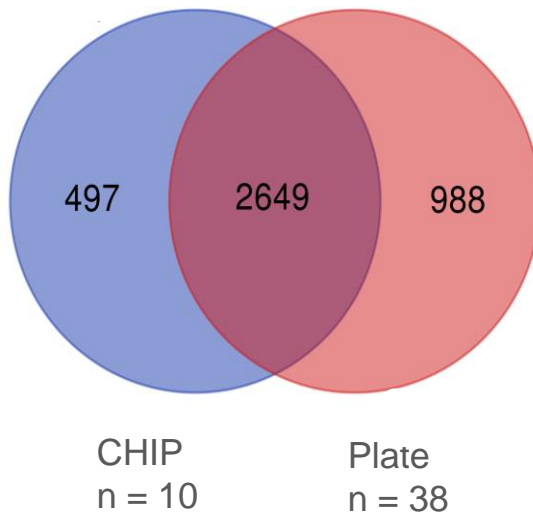




## Chip

#term ID	term description	observed gene count	FDR
GO:0097458	neuron part	83	7.43E-10
GO:0043005	neuron projection	69	5.68E-09
GO:0120025	plasma membrane bounded cell projection	96	6.89E-09
GO:0042995	cell projection	98	6.91E-09
GO:0120025	plasma membrane bounded cell projection	96	6.89E-09
GO:0042995	cell projection	98	6.91E-09
GO:0045202	synapse	51	1.38E-06
GO:0120038	plasma membrane bounded cell projection part	66	6.69E-06
GO:0030424	axon	36	8.75E-06
GO:0071944	cell periphery	183	1.58E-05
GO:0005886	plasma membrane	178	4.62E-05
GO:0036477	somatodendritic compartment	41	1.10E-04
GO:0016020	membrane	259	3.50E-04
GO:0030425	dendrite	32	3.50E-04
GO:0044456	synapse part	38	5.10E-04
GO:0031982	vesicle	90	7.10E-04
GO:0098805	whole membrane	66	7.90E-04
GO:0098794	postsynapse	26	2.10E-03
GO:0030427	site of polarized growth	14	3.20E-03
GO:0031410	cytoplasmic vesicle	84	3.20E-03
GO:0015630	microtubule cytoskeleton	49	3.80E-03
GO:0098552	side of membrane	25	4.40E-03
GO:0098588	bounding membrane of organelle	74	6.20E-03
GO:0030426	growth cone	13	7.10E-03

## Proteomic DIA-MS



## Plate

#term ID	term description	observed gene count	FDR
GO:0043231	intracellular membrane-bounded organelle	734	1.68E-44
GO:0070013	intracellular organelle lumen	468	1.02E-43
GO:0043227	membrane-bounded organelle	764	1.31E-41
GO:0044428	nuclear part	393	1.07E-33
GO:0031981	nuclear lumen	370	1.26E-32
GO:0005634	nucleus	524	4.65E-30
GO:0005654	nucleoplasm	324	3.31E-29
GO:0032991	protein-containing complex	387	1.61E-23
GO:0043232	intracellular non-membrane-bounded organelle	334	1.71E-21
GO:1990904	ribonucleoprotein complex	108	1.01E-18
GO:0005730	nucleolus	113	1.52E-15
GO:0030684	preribosome	29	9.30E-14
GO:1990904	ribonucleoprotein complex	108	1.01E-18
GO:0005730	nucleolus	113	1.52E-15
GO:0030684	preribosome	29	9.30E-14