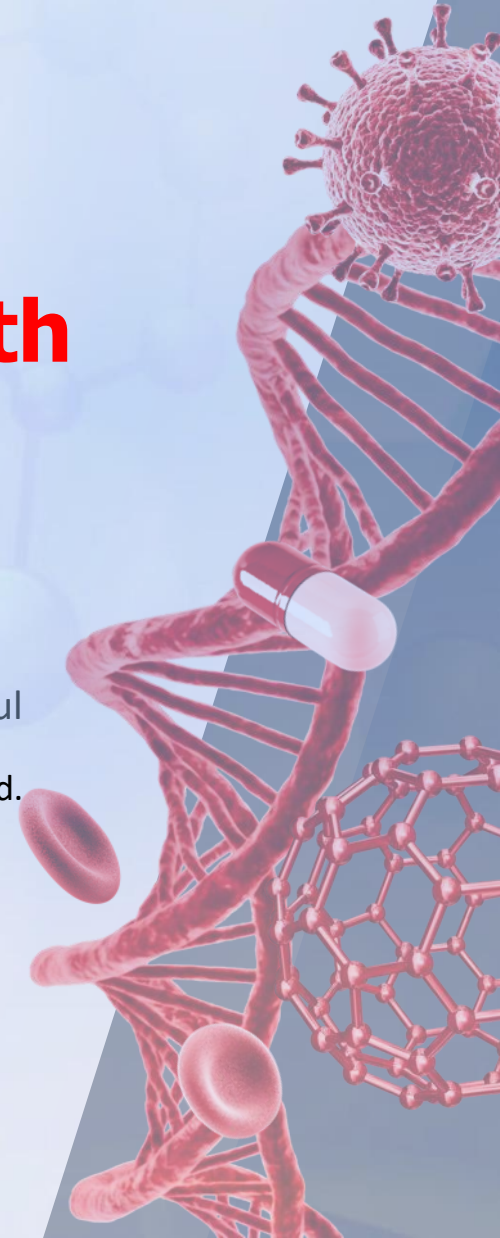


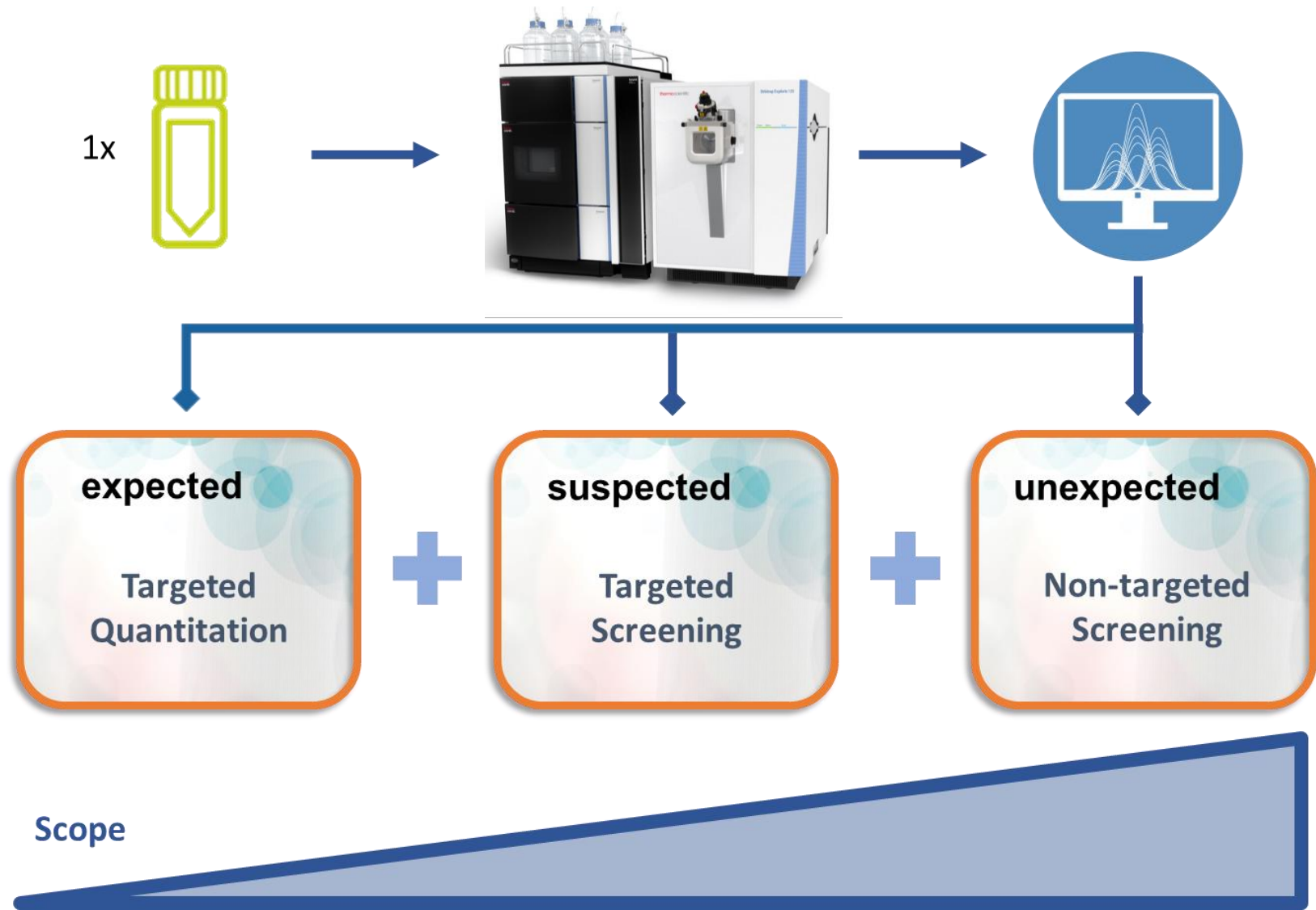
# Current Trends in Clinical Diagnosis with Orbitrap High-Resolution Mass Spectrometry-based Techniques

Jitnapa Voranitikul

Product Specialist, SciSpec Co., Ltd.



# System Main Workflows



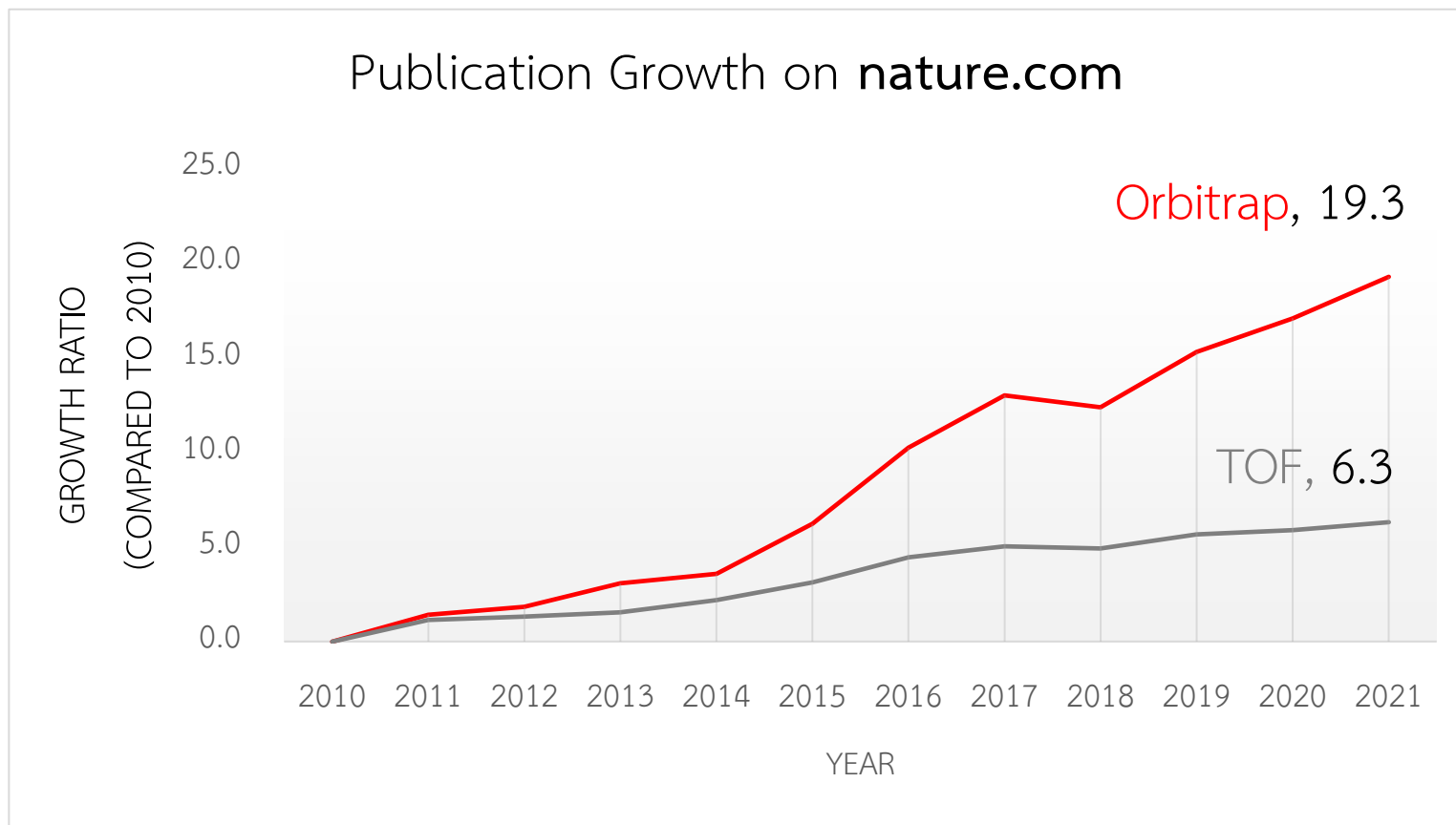
*Thermo Scientific*

*Orbitrap HRAMS System*



# Mass Analyzer : Orbitrap™ Technology

Year	Orbitrap	TOF
2010	61	165
2011	87	189
2012	112	219
2013	188	255
2014	219	361
2015	380	516
2016	625	734
2017	794	830
2018	755	813
2019	934	934
2020	1042	971
2021	1176	1042
<b>Total</b>	<b>6373</b>	<b>7029</b>



By the end of 2021, the publication ratios in each years, compared to those of 2010, show superiority of the publication growth from Orbitrap technology 3 times higher than that of TOF.

## Application

- Endocrinology – IGF-1
- Metabolic syndromes – IEM hemoglobinopathy
- Precision medicine & advanced clinical diagnostics
  - Infectious disease (Ucalgary, Nanopin, Fleury), pandemic responsiveness
  - Alzheimer's disease C2N, Reproductive Health NX Prenatal

# Quest Diagnostics: IGF-1 LCMS assay



Quantification of insulin-like growth factor 1 in human serum by Vanquish UHPLC with Q Exactive high-resolution accurate-mass mass spectrometry for clinical research

- Quantitation and identifying variants of IGF-1 using high resolution mass spectrometry.

Identification of IGF-1 variants helps support physician's decisions when treating growth-related patients.

- Development of a robust protein assay by "top-down" measurement of IGF-1 in human serum
- Improve assay performance using HRAM MS
- Identify IGF-1 variants and understanding its clinical significance

Sample (serum)

Step 1; Acidic ethanol precipitation and neutralization pre-cooling

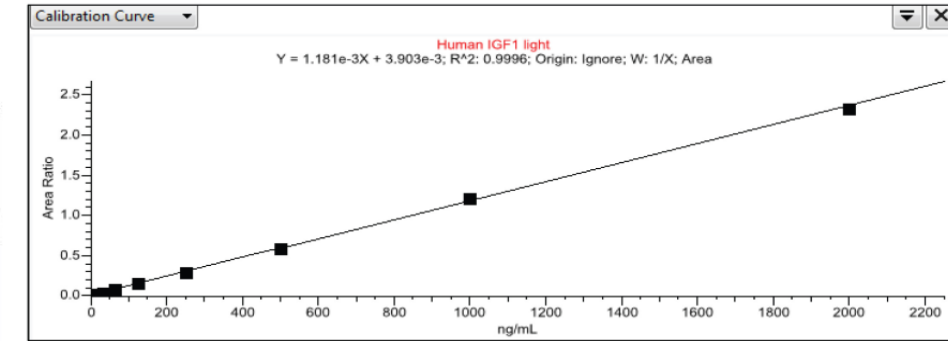
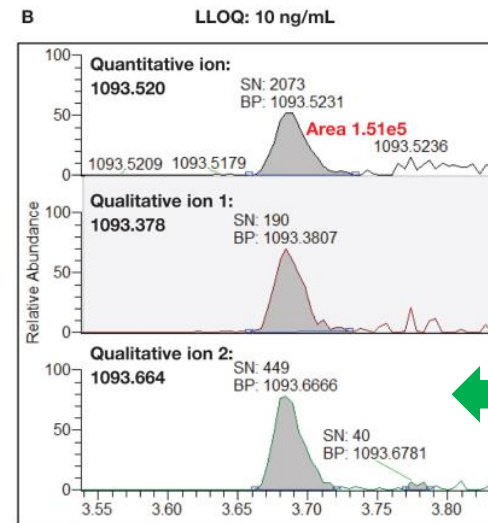
To eliminate the interference of IGFBP3

Step 2; Online SPE

To eliminate matrix

Step 3; LC-MSMS

Vanquish UHPLC + Q Exactive Focus HRAM MS



Analyte	Linearity range	r <sup>2</sup> linear fit, 1/x weight	LLOQ	Internal standard CV%
Human IGF-1	15.6–2000 ng/mL	0.9996	10 ng/mL	3.96

The quantitative and qualitative ions at the LLOQ (10 ng/mL) still present a good chromatographic peak shape and an excellent S/N ratio. (The lower limit of the ref range is 14 ng/mL)

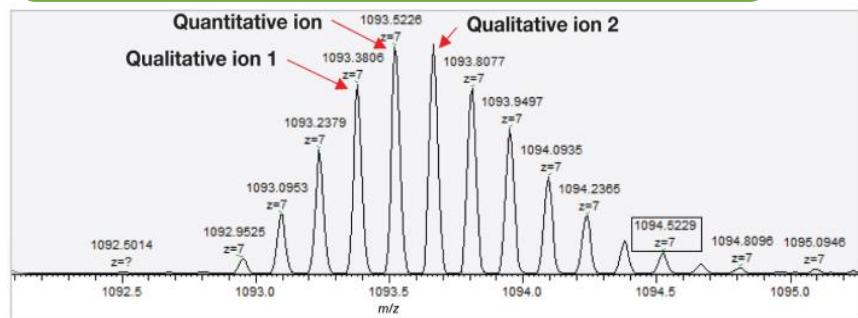


Figure 4. Chromatography mass spectra for insulin-like growth factor quantitative, qualitative, and internal standard ions

## Outstanding of Benefits;



- Established reference range comparable to gold standard immunoassay method
- Better sensitivity, specificity vs immunoassay approach (the main way to measure IGF-1)
- Developed an effective and high-throughput method to monitor IGF-1 and its variants using high resolution LC-MS coupled with online extraction in a clinical laboratory
- High resolution MS revealed the complexity of IGF-1 variants

# Case Study: Archimed Life (2016 – 2019)

HRAM/MS method fits ideal requirements for Clinical

- Collaborator: David Kasper (Founder), Thomas Wiesinger
- Implementation of novel HRAM/MS methods for Newborn screening on **Q Exactive Focus MS**
  - Prospective study of ~5000 de-identified newborn samples (UKE Hamburg) – Screening of hemoglobinopathy and thalassemia
  - Applicability of HRAM/MS in untargeted screening protocol for rare mutations

Thomas Wiesinger, Thomas Mechtler, Markus Schwarz, Xiaolei Xie, Regine Grosse, Paulina Nieves Cobos, David Kasper\* and Zoltan Lukacs\*  
**Investigating the suitability of high-resolution mass spectrometry for newborn screening: identification of hemoglobinopathies and  $\beta$ -thalassemias in dried blood spots**

<https://doi.org/10.1515/cclm-2019-0832>  
 Received August 8, 2019; accepted December 23, 2019; previously published online February 6, 2020

## Introduction



### The major challenge for a screening assay

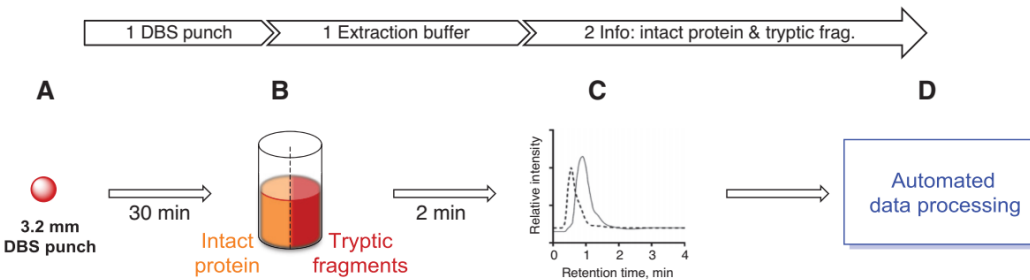
- ❑ Identification and differentiation between carrier- and sickle cell disease-positive samples
- ❑ Single mutation of clinical relevance in the beta-chain (e.g. Hb C, D, E), these Hb variants differ only by 1 Da for the entire protein.

“The method is suitable for the identification of clinically relevant mutations and differentiation between hetero, homozygous form”



### Outstanding of Benefits:

- ✓ Minimal sample preparation and handling
- ✓ High throughput (2 min/sample)
- ✓ Inexpensive (6–7 € Euro/sample)
- ✓ Robust system (no maintenance – only one time source cleaning during the study)
- ✓ Automated clinical assessment



**Figure 1:** Illustration of the general workflow. (A) Single DBS punch extracted with tryptic buffer (40 mM  $\text{NH}_4\text{HCO}_3$ , 9% MeCN, 1 mM  $\text{CaCl}_2$ , 5 mg/mL TPCK-treated trypsin from bovine pancreas) for 30 min; (B) mixture of the intact protein ( $\alpha$ ,  $\beta$  and  $\gamma$ ) and tryptic fragments (e.g. C, D, E and S); (C) flow injection (duplex mode) is performed after quenching (2 min/sample); (D) results are processed with TraceFinder™.

<https://www.degruyter.com/document/doi/10.1515/cclm-2019-0832/html>

RESULTS OF THE PROSPECTIVE STUDY



**Variant II**

UKE Hamburg  
Eppendorf

Summary of the Prospective Study:  
 Hemoglobinopathies & Thalassemias by HRMS

	Variant II	HRMS	HRMS 2 <sup>nd</sup> Tier Testing	Genetic Testing
FAS	35	35	-	n.p.
FAC	9	9	-	n.p.
FAD	8	8	-	n.p.
FAE	4	1	-	*
$\beta$ Thal	0	4	1	*
Hb Presbyterien	0	1	1	*
Hb unknown	0	1	0	*
Not found	1 (516)	-	*	Not found

\*Performed by the UKE Hamburg (pending)



**HRMS**

Archimedlife  
Science GmbH

Beta Thal suspected by the screening method

## Automated Dried Spot Analysis –

Comprehensive workflows in one platform

 The world leader in serving science



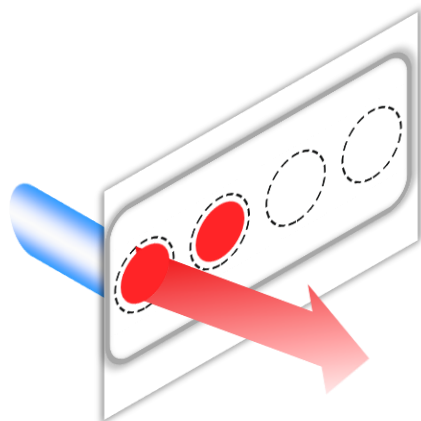


# What is Flow-Through Desorption?



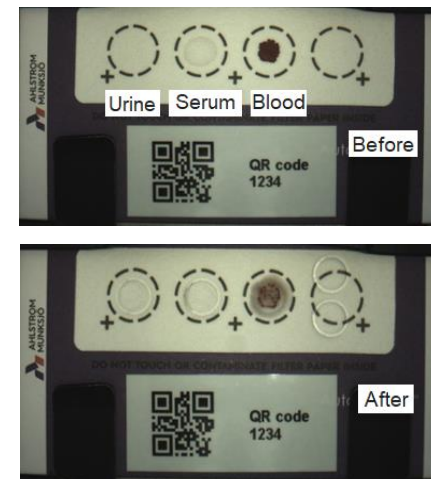
## Dried Matrix Spot Cards

- Whatman DMPK
- PerkinElmer 226
- Ahlstrom Autocollect
- HemaXis DB10



## FTD™ Flow-Through Desorption Technology

- No disc-punching – Direct elution of DBS from cards
- Leak-tight clamp heads
- Highly efficient desorption



## Dried Matrix Sent Directly to LC/MS

- Flow injection
- Analytical Flow
- Turboflow

\* Patented

\* FTD is a trademark of Spark Holland

# Tuberculosis (TB) by NanoPin's Technology

NanoPin uses an antibody enrichment approach to capture species-specific biomarker peptides.



May 21, 2020

Desert Platforms MDC  
Rahul Rao  
Project Manager  
275 N. Gateway Dr.  
Phoenix, Arizona 85034

Re: Q191789/S001  
Trade/Device Name: NanoDetect-TB  
Received: February 26, 2020

Dear Rahul Rao:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has received the above submission requesting designation as a Breakthrough Device. The proposed indications for use includes "NanoDetect-TB is indicated for use in the detection of active TB disease. It is an in-vitro diagnostic test for the direct detection of target peptides derived from proteins secreted by Mtb bacilli into the circulation, which are then assayed in serum or plasma samples collected by standard intravenous blood draws. Antibody-conjugated nanoparticles are employed to enrich the target peptides from trypsin digested serum or plasma samples, which are then analyzed by MS to report a positive or negative result for active TB disease. The intended use population are symptomatic patients suspected to have active TB disease and high-risk individuals." We are pleased to inform you that your device and proposed indication for use meet the criteria and have been granted designation as a Breakthrough Device. Please refer to the FDA guidance document entitled "Breakthrough Devices Program", for more information regarding the program, available at <https://www.fda.gov/media/108135/download>.

We recommend you review the FDA guidance document for the Breakthrough Devices Program referenced above for the available mechanisms for obtaining feedback from the Agency on device development for designated breakthrough devices. When submitting any new requests, please reference Q191789/S001. Any new submission should be provided as an eCopy, it should include the FDA reference number for this submission, and should be submitted to the following address:

U.S. Food and Drug Administration  
Center for Devices and Radiological Health  
IDE Document Control Center - WO66-G609  
10903 New Hampshire Avenue  
Silver Spring, MD 20993-0002



Prof. Tony Hu  
Tulane University | Co-Founder  
of NanoPin Technologies, Inc.

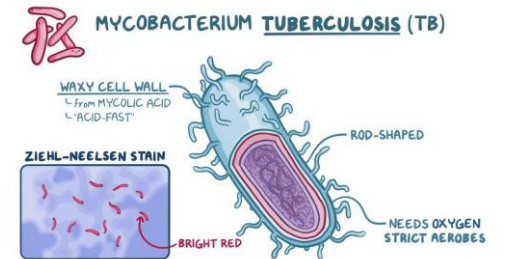


## Pulmonary and ExtraPulmonary Detection



### Main sites of Extrapulmonary tuberculosis

- Central nervous system** - Meningitis
- Lymphatics** - Scrofula (of the neck)
- Pleura** - Tuberculosis pleurisy
- Disseminated** - Miliary tuberculosis
- Bones and joints of spine** - Pott's disease
- Genito-urinary** - Urogenital tuberculosis



Selection of target peptide from the untargeted analysis.

HRMS (Orbitrap)



target peptide

TSQ (Altis)

Current method

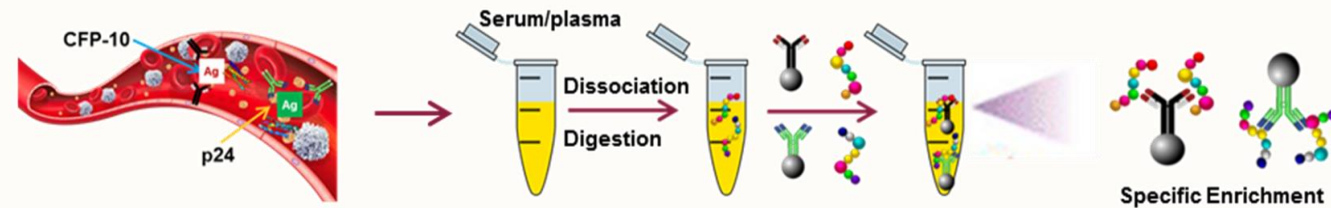
New Assay (NanoPin)


Tissue biopsy

Serum


# Tuberculosis (TB) by NanoPin's Technology

- Collaboration press release ([link](#))
- Support upcoming clinical trial strategies in US and China
- **Infectious disease diagnostics \$26B** with TB Segment >\$3 billion by 2024 and HIV Segment \$5 billion by 2023



Challenge: 

- ❖ Low concentration in the bloodstream (Especially early in infection)
- ❖ Disturb by abundant serum protein
- ❖ Conserved protein by closely related pathogens

Benefits from digestion: 


- ❖ Remove the masking effect
- ❖ Increase the amount of target peptide
- ❖ Distinguish highly conserved proteins by closely related pathogens

**Conclusion**

- Antibody-mediated enrichment of target peptides prior to their analysis by LC-MS/MS

**Workflow**

- R&D discovery on Thermo Fisher Orbitrap and triple quadrupole MS
- Successfully transitioned and implemented NanoPin TB assay on Vanquish MD + TSQ Altis MD

**Advantages of the alternative method** 

- ✓ High increase in both sensitivity and specificity
- ✓ No tissue biopsy
- ✓ Quick response (LC-MS/MS)

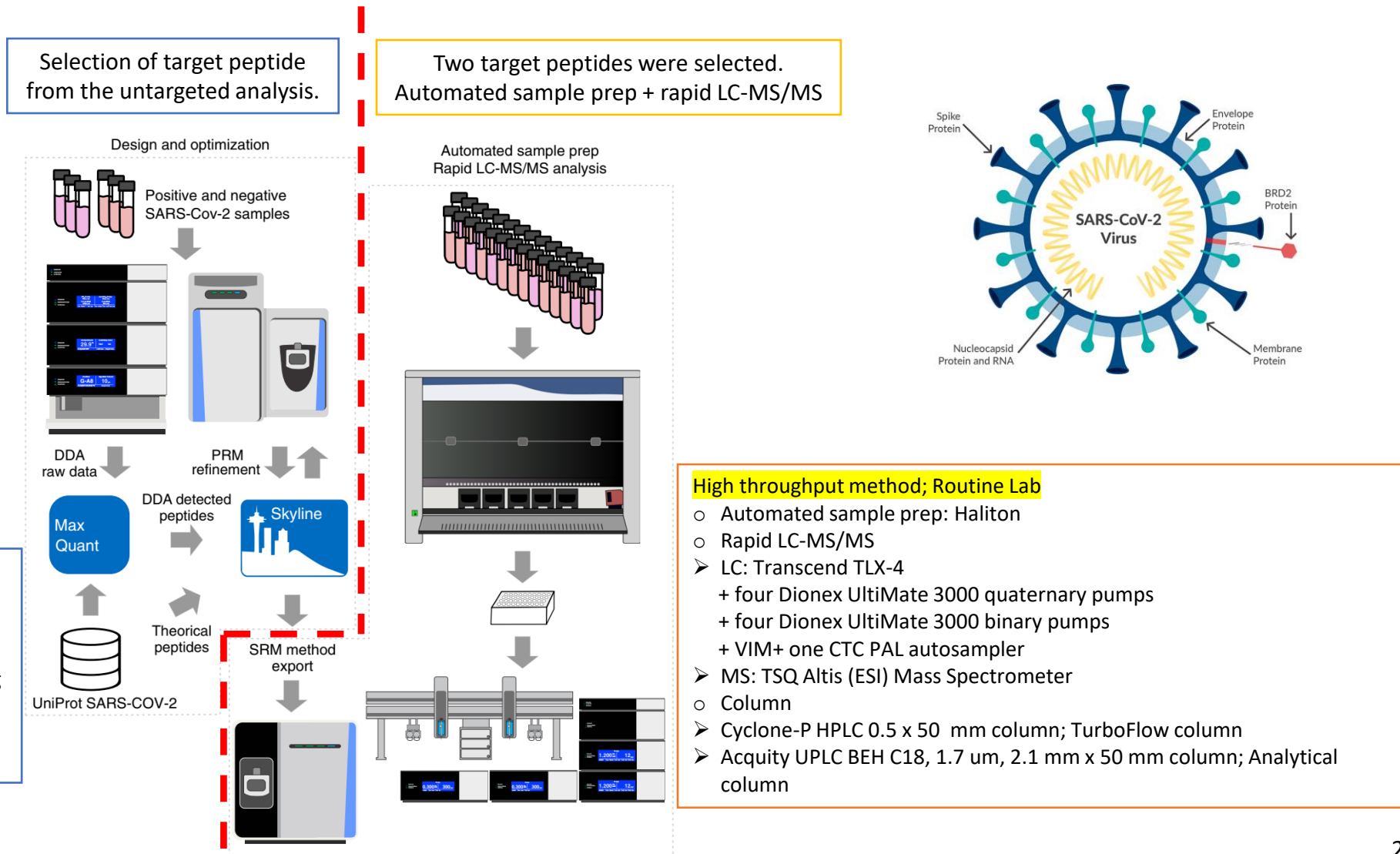
## Establishing a mass spectrometry-based system for rapid detection of SARS-CoV-2 in large clinical sample cohorts.



**Dr. Valdemir Carvalho**  
Flcury Group, Brazil

### Develop Phase

- LC: Ultimate 3000 Nano
- MS: Q-Exactive HF-X Mass spectrometer
- Column
- PepMap 100 C18, 5 um, 0.3 x 5 mm; sample trapping precolumn
- PepMap RSLC C18, 2 um, 150 um x 15 cm; analytical column



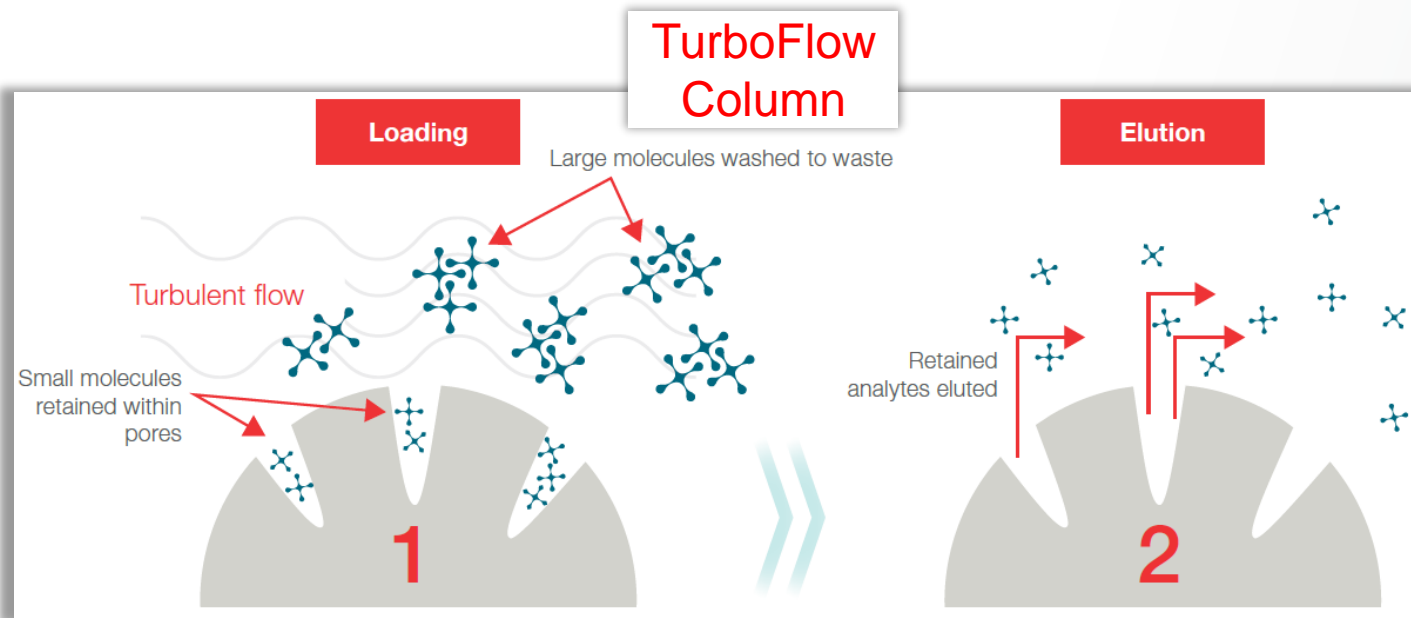
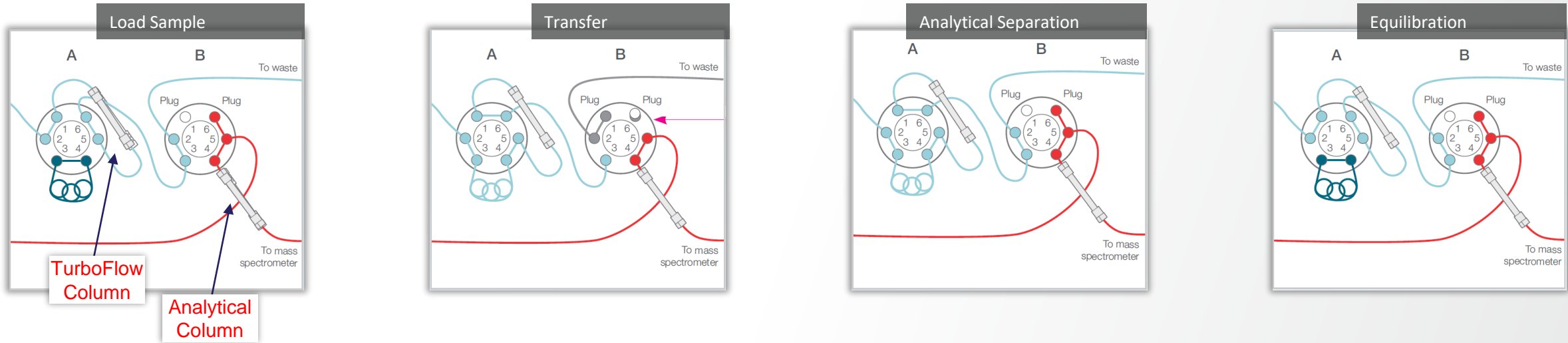
# Transcend II TLX-1 System

- Flow injection (FIA)
- LX Mode – Analytical Flow
- TLX Mode – TurboFlow
- Control and timing controlled by Aria MX Software

- Pressure – 1000 bar
- Viper Fingertight Fittings
- Features a biocompatible 2 channel binary high-pressure gradient mixing pump with 2 x 3 solvent channels. Select among 3 solvents per piston assembly (A1, A2, A3 & B1, B2, B3)



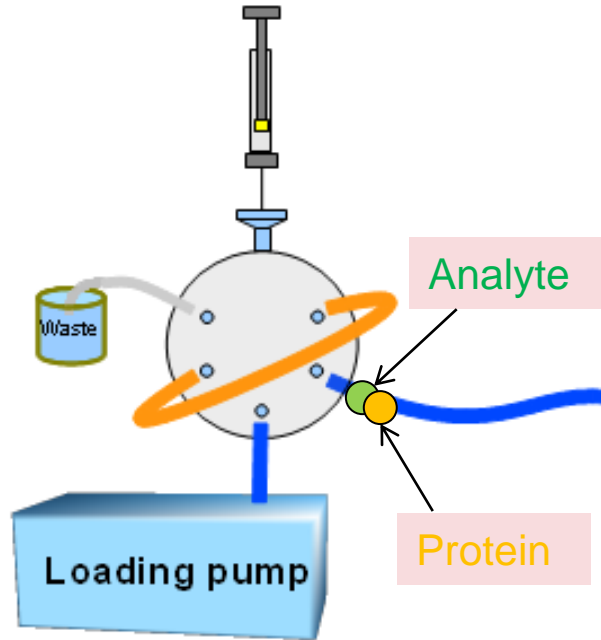
# TurboFlow Technology



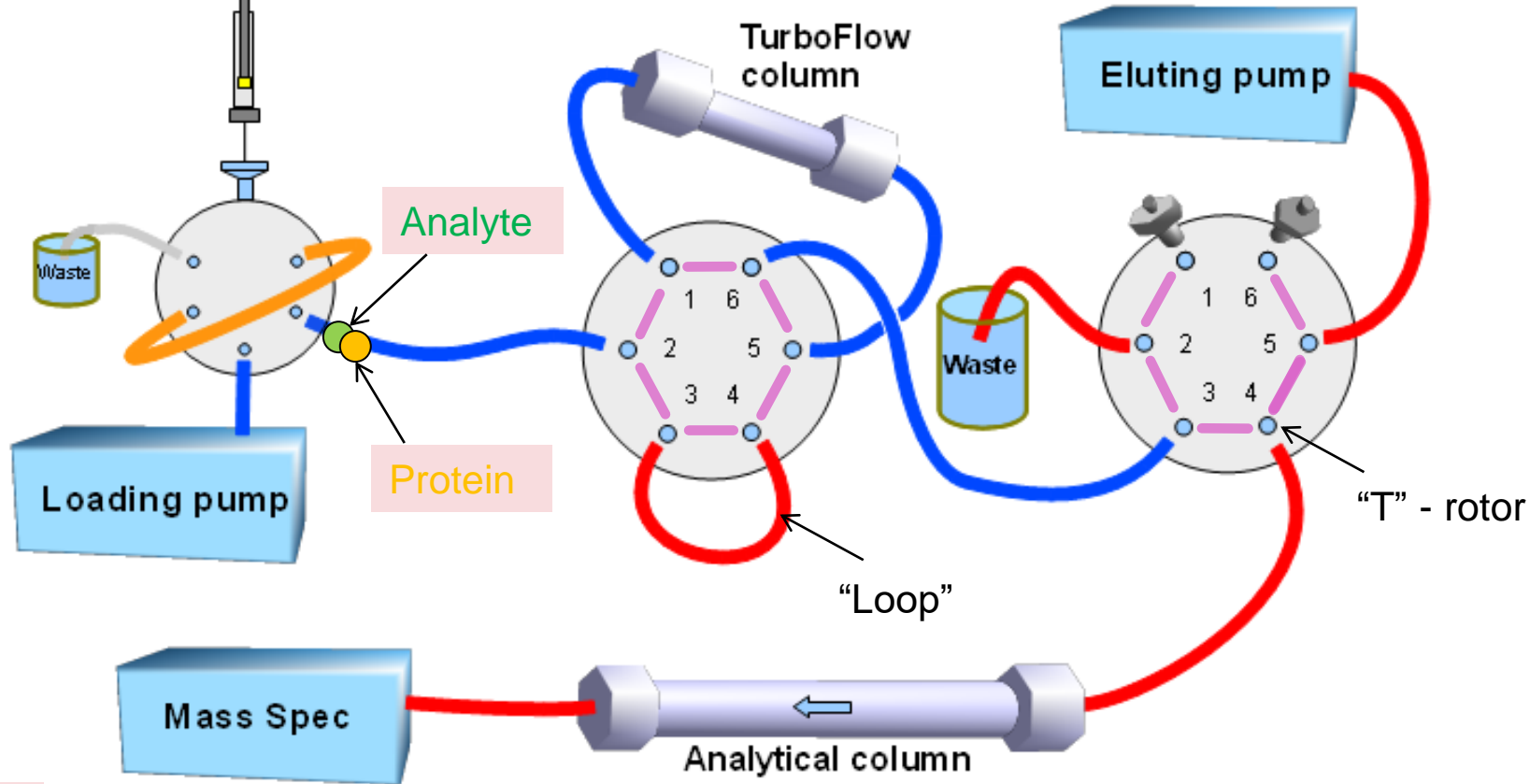
- Small molecules diffuse into porous particles faster than large molecules
- Sample components of interest - Analytes - are well retained
- Less retained components (e.g., salts & sugars) are rinsed away

# TurboFlow Focus Mode Plumbing

## 1. Loading

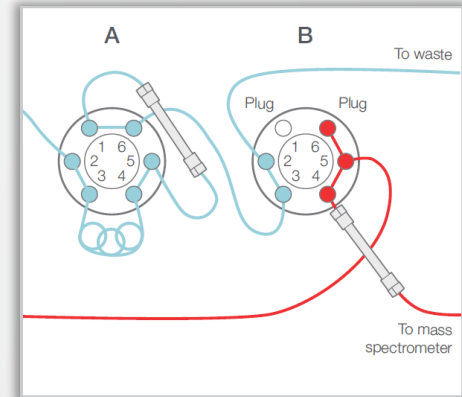


## 2. Transfer – Elution and Focusing

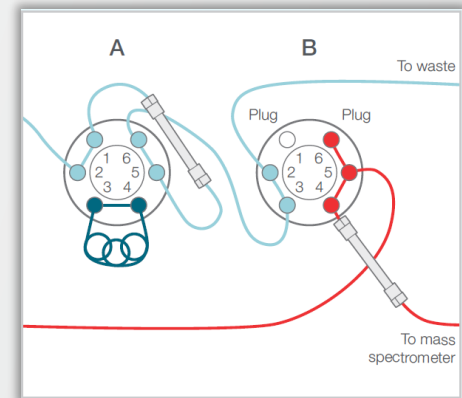


- Analyte
- Protein

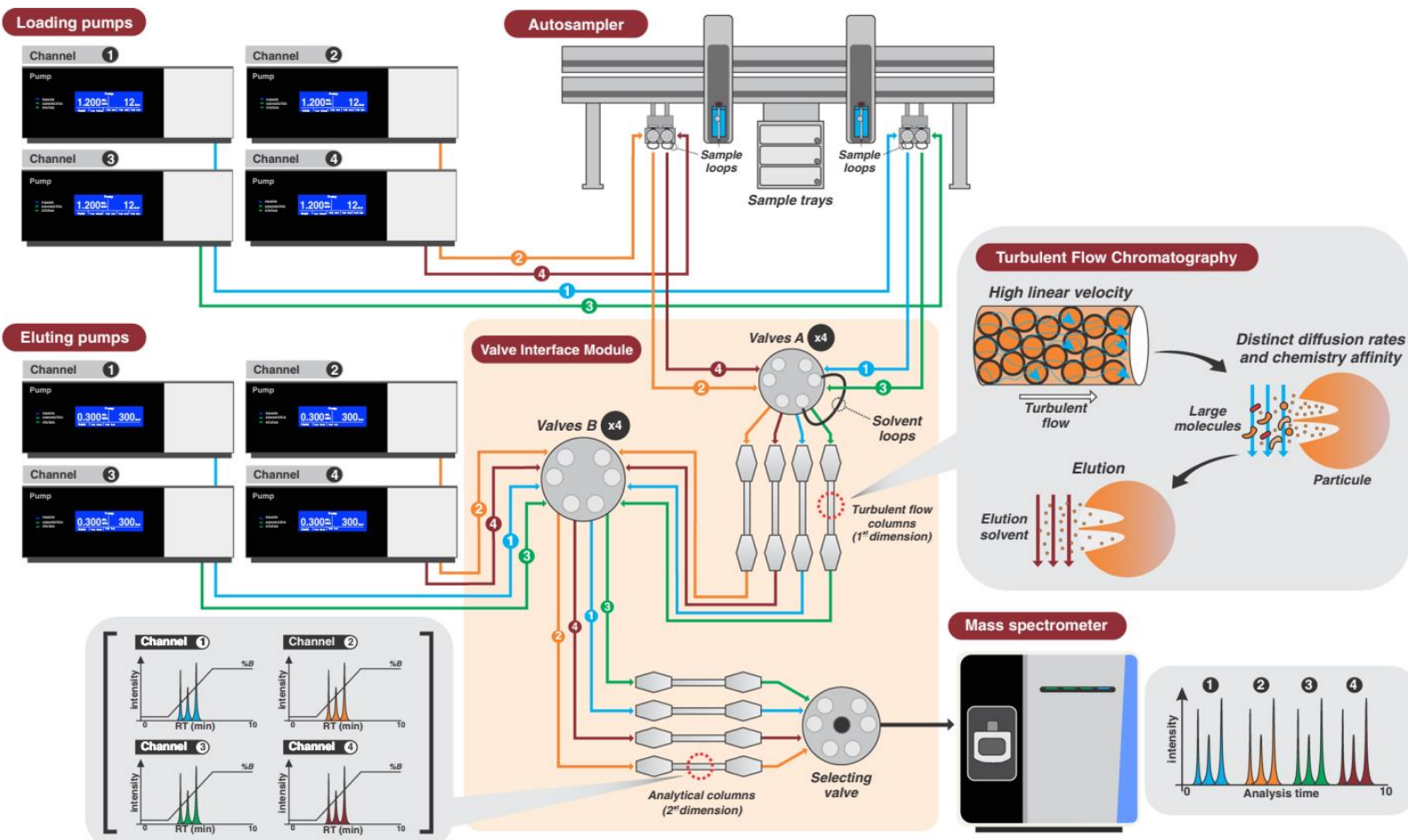
## 3. TX column cleaning LC column gradient



## 4. Re-conditioning



The concept application of automated sample preparation and multiplexing TFC coupled to triple quadrupole MS as a feasible alternative for detecting SARS-CoV-2 in clinical respiratory tract samples on a large scale at a population, level was proved.



## Conclusion

- ✓ High throughput targeted proteomics assay i.e., LCMS approach as an alternative testing strategy for large populations

## Outstanding of Benefits

- ✓ Analysis resulted in a 2.5-min acquisition time per sample (96 sample/4h ~500 samples/day)
- ✓ Circumvent reagent shortages
- ✓ Better specificity vs. immunoassay approach (standard method)

- Automated sample prep
- Rapid LC-MS/MS
- LC: Transcend TLX-4
  - + four Dionex UltiMate 3000 quaternary pumps
  - + four Dionex UltiMate 3000 binary pumps
  - + VIM+ one CTC PAL autosampler
- MS: TSQ Altis (ESI) Mass Spectrometer
- Column
  - Cyclone-P HPLC 0.5 x 50 mm column; TurboFlow column
  - Acquity UPLC BEH C18, 1.7 um, 2.1 mm x 50 mm column; Analytical column



Fig. Schematic illustration of turbulent flow chromatography (TFC) setup in Transcend TLX-4 system coupled to TSQ Altis triple quadrupole mass spectrometer



C<sub>2</sub>N Diagnostics Introduces the PrecivityAD<sup>2</sup><sup>™</sup> Blood Test  
Next Generation Blood Test Aims to Establish New Standard  
in Alzheimer's Disease Diagnosis with Combined Measures  
of Amyloid Beta and Tau Protein

1st precise clinical Alzheimer's blood test on Thermo Fisher Orbitrap Lumos Tribid MS

## Objectives

- Develop a LCMS approach to better diagnose and treat Alzheimer's and other neurodegenerative diseases
- **Non-invasive, less expensive and scalable**
- Need for amyloid tests as **companion diagnostics** for AD treatment

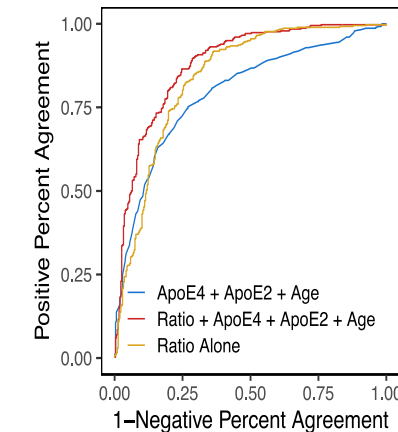
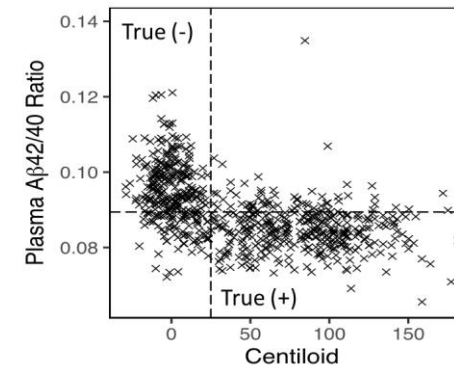
## Deliverables & Impact

- Thermo Fisher-enabled platform for early brain pathology detection and early treatment
- **Address public health burden, \$600B** blood-based AD diagnostics, health and wellness market and companion diagnostics for AD treatment
- **Deployment of IVD kits** to qualified reference labs globally



PrecivityAD was granted designation as a *Breakthrough Device* by FDA in 2018

- ✓ PrecivityAD<sup>™</sup> Performance – High Correlation to PET scan results
- ✓ Positioning PrecivityAD<sup>™</sup> as a Rule In/Rule Out Amyloid test for ~80% of the intended use patient population
- Comparison between PrecivityAD<sup>™</sup> test and amyloid PET scan results (+ PET defined by centiloid >25)



AUC  
0.79  
0.88  
0.84

Source: C<sub>2</sub>N Diagnostics. A mass spectrometry-based blood diagnostic test measuring plasma Ab42/40 ratio, APOE proteotype and combined with age, identifies brain amyloid pathology: a clinical validity study

# Sci Spec Rapid infections diagnostics: University of Calgary

- Thermo Fisher-enabled platform for rapid, metabolomics-based diagnostics approach for infectious disease

## Hopper™ | Rapid diagnostic platform for identification and antibiotic susceptibility testing of pathogens



**Ian Lewis, PhD; Associate Professor** *Lewis Research Group, UCalgary MS lab*  
 AI Translational Health Chair *showcase at the ACAD grand opening*  
 University of Calgary

ARTICLE Check for updates  
<https://doi.org/10.1038/s41467-022-30048-6> OPEN  
**Metabolic preference assay for rapid diagnosis of bloodstream infections**  
 Thomas Rydzak<sup>1</sup>, Ryan A. Groves<sup>1</sup>, Ruichuan Zhang<sup>1</sup>, Raied Aburashed<sup>2</sup>, Rajnigandha Pushpker<sup>1</sup>, Maryam Mapar<sup>1</sup> & Ian A. Lewis<sup>1</sup> <sup>1</sup>✉

	Sample preparation (incubated period)	Method
Current method	2-5 days	MALDI-TOF (DI), AST
New method	4 h (MPA)	LC-MS/MS

MPA = Metabolic preference assay; metabolic fluxes observed in ex-vivo

**Conclusion**

- ✓ Diagnostic platform for implementing high-volume diagnostic tests for bloodstream
- ✓ Rapidly identify microorganisms and measuring their antibiotic susceptibility profiles (AST) using a LCMS approach

**Workflow**

- ✓ R & D discovery on Thermo Fisher Orbitrap (Q Exactive HF MS (negative)-MAVEN (SW))
- ✓ Successfully established the use of Vanquish Flex+ TSQ Altis MS-TraceFinder software for microbial identification and antimicrobial susceptibility testing (AST)



- Potential implementation into DynaLIFE – multiple reference sites across Alberta and Canada

**Outstanding of Benefits**

- ✓ Replaces standard tests performed by MALDI-TOF (ID) and VITEK (AST)
- ✓ Reduces current microbiology testing workflow form 2-5 days to 4 hours

## Addressing a critical clinical unmet need in women's health

NX Prenatal Announces Publication of Peer-Reviewed Study in the American Journal of Obstetrics & Gynecology for Validation of First Trimester Preterm Birth Risk Biomarkers

The findings represent the first reported multi-site validation of blood-based biomarker panels at 10-12 weeks gestation to stratify pregnant patients for the risk of preterm birth

Feb 28, 2019, 12:18 ET

LOUISVILLE, Ky. and HOUSTON, Feb. 28, 2019 /PRNewswire/ -- NX Prenatal Inc., focused on development of proprietary blood-based, early warning molecular diagnostic tests for adverse pregnancy outcomes, announced today the publication of a peer-reviewed study, "Circulating Microparticle Proteins Obtained in the Late First Trimester Predict Spontaneous Preterm Birth at Less than 35 Weeks Gestation: A Panel Validation with Specific Characterization by Parity" in the American Journal of Obstetrics & Gynecology. The publication is available online: <https://doi.org/10.1016/j.ajog.2019.01.220>.

### Objectives

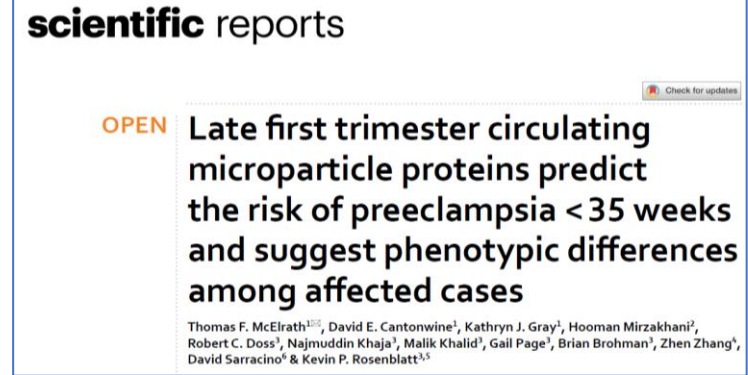
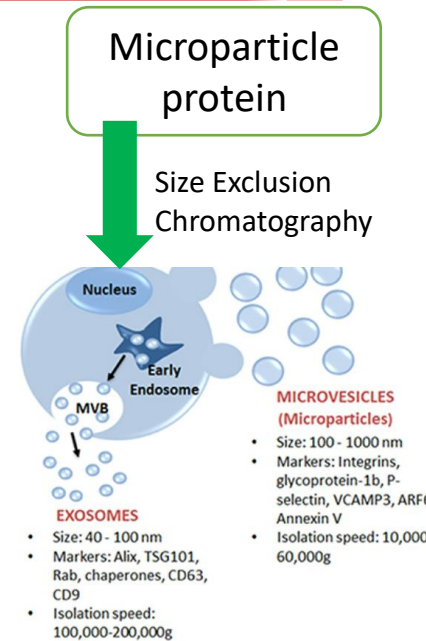
- Exosome isolation analytical approach for targeted biomarker panel development

### Conclusion

- ✓ Blood test for assessing the risk of pre-term birth as easily as 10-12 weeks (compatible with NIPT testing)

### Workflow

- ✓ R & D discovery on Thermo Fisher Orbitrap Fusion Tribrid
- ✓ Successfully transitioned and implemented targeted peptide biomarker panel on LC-MS/MS Vanquish Horizon UHPLC + TSQ Altis MS



TurboFlow + Analytical Column  
+ **Fusion Lumos Orbitrap**

Peptide spectrum library

Target peptide biomarker panel

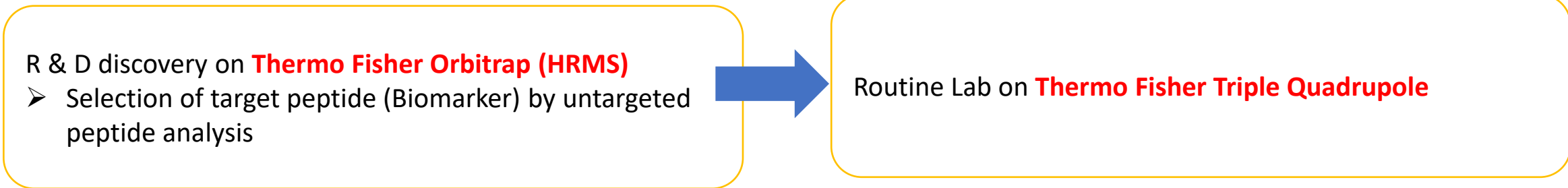
Transfer Method  
→ **LC (Vanquish Horizon)**  
+ **TSQ (Altis)**

### Outstanding of Benefits

- ✓ Quick access to prophylaxis and therapy
- ✓ Reduce NICU dependency
- ✓ Enabled development of new pipeline product (Placenta Accreta Study)

	Benefit of using HRMS
<ul style="list-style-type: none"> <li>• <b>Endocrinology – IGF-1</b></li> </ul>	<ol style="list-style-type: none"> <li>1. High sensitivity and selectivity vs immunoassay approach</li> <li>2. Reveal complex of IGF-1 variants</li> <li>3. High throughput</li> </ol>
<ul style="list-style-type: none"> <li>• <b>Metabolic syndromes – IEM hemoglobinopathy</b></li> </ul>	<ol style="list-style-type: none"> <li>1. Identification and differentiation of carrier and sickle cell disease-positive in beta chain</li> <li>2. Single mutation of clinical relevance</li> </ol>

- **Precision medicine & advanced clinical diagnostics**  
 Infectious disease (Ucalgary, Nanopin, Fleury), pandemic responsiveness  
 Alzheimer’s disease C2N, Reproductive Health NX Prenatal



- ✓ Adding some sample preparation technology ex. Nanopin technology for TB assay
- ✓ Adding some automated system ex. Online SPE, Transcended TLX 4 which supports high throughput targeted peptide (Biomarker) assay

# ติดตามกิจกรรมของบริษัทได้ที่



[www.scispec.co.th](http://www.scispec.co.th)



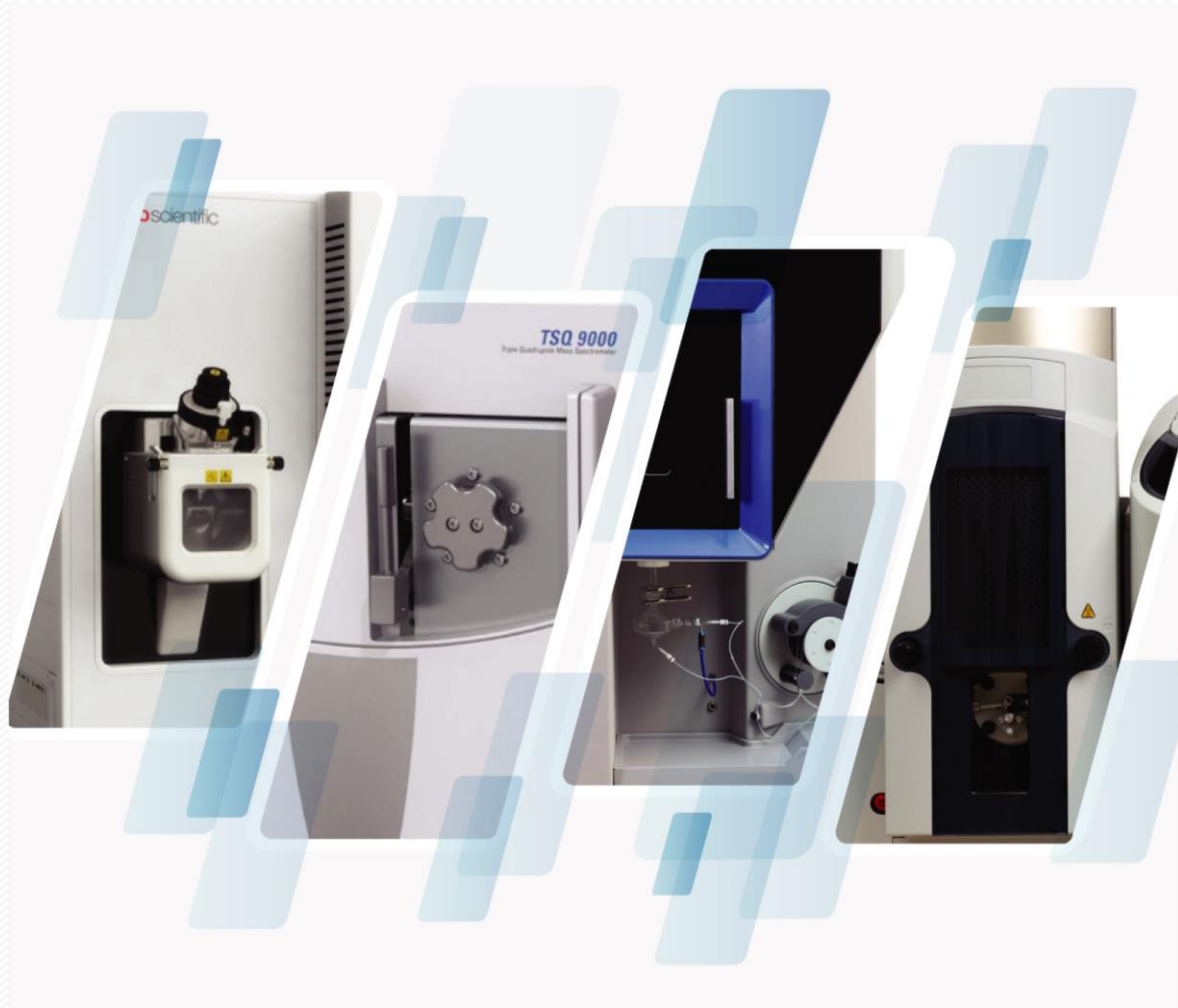
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[@scispec](https://line.me/tv/@scispec)



[crm@scispec.co.th](mailto:crm@scispec.co.th)



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SCIENTIFIC

**MARKES**  
international



**GAS**

**CTC Analytics**



**908devices**

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