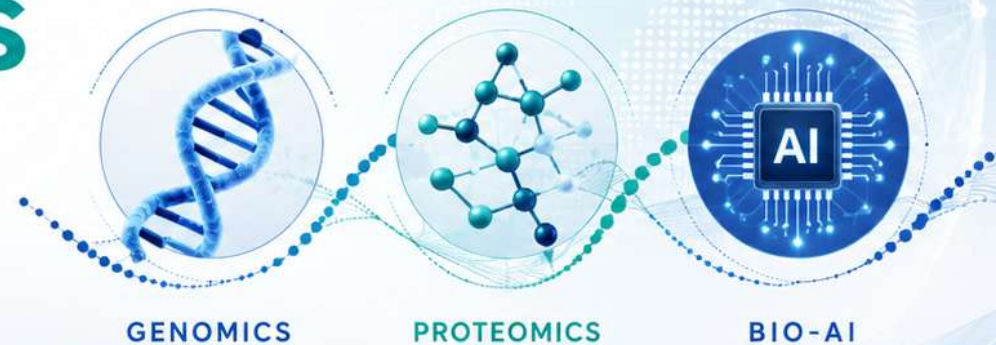


Building the Future of Clinical Omics

Integrating Genomics, Proteomics and Bio-AI





INLab Precision Medicine Solution Provider



Global R&D Headquarters: Nangang National Biotechnology Research Park, Taipei, Taiwan
Driving innovation in molecular diagnostics and precision medicine solutions.



European Operations: Utrecht, The Netherlands
Including warehouse facility in Almere to support seamless logistics and customer service across Europe.



US Business Development Laboratory: New York, USA
Advancing partnerships and clinical applications in the North American market.



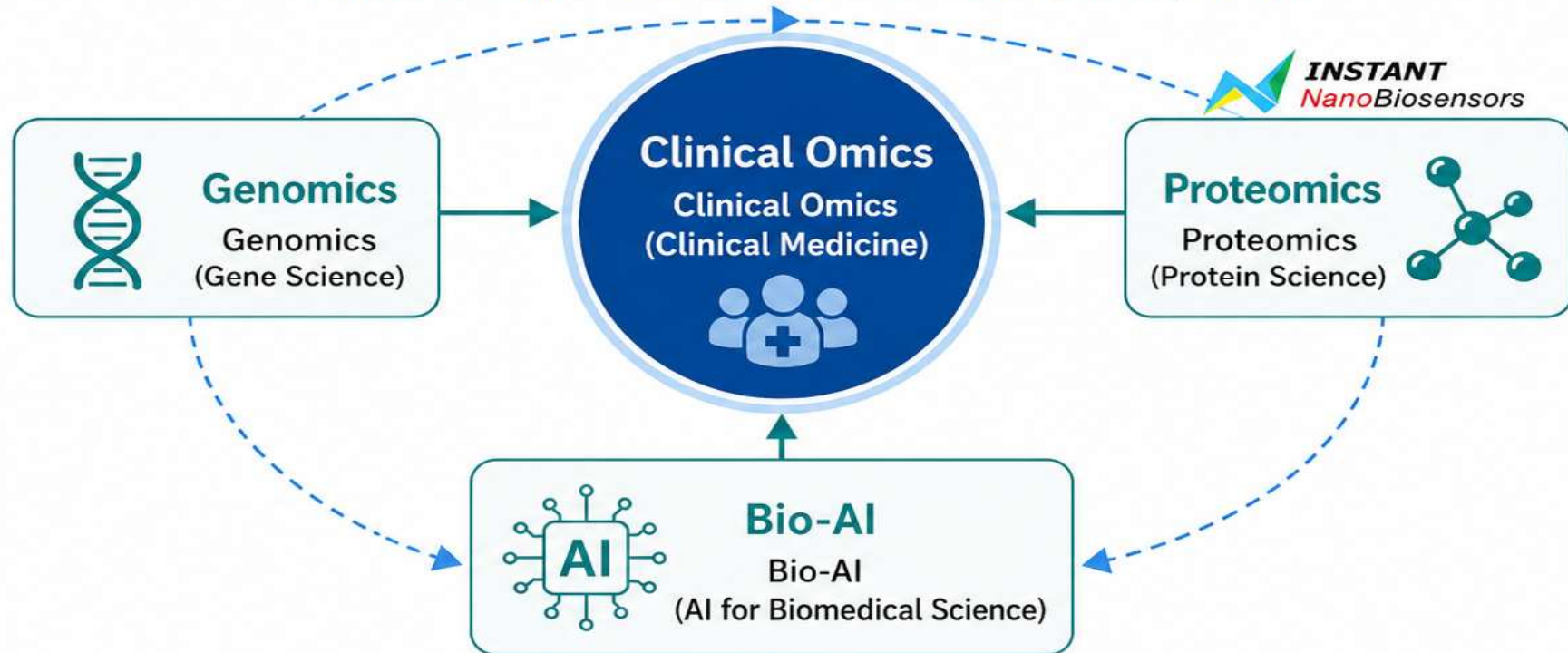
Manufacturing Excellence: ISO 13485—certified cleanroom facility in Xizhi, Taiwan
Built with an integrated Quality Management System (QMS) to ensure consistent quality and regulatory compliance.

Global Presence. Local Commitment. Precision for a Healthier Future.



INB DEVELOPMENT ROADMAP

Clinical Omics at the Core of Our Development



Integrating Genomics, Proteomics, and Bio-AI,
Driving the Next Generation of **Clinical Omics** and **Precision Medicine**

iNA INLab NGS Precision Medicine

Sample-to-Report Integrated Workflow



Automation

Streamlined workflows for higher efficiency and consistency.



Standardization

Validated processes and quality by design for reproducible results.



Deployability

Built for in-house implementation and real-world impact.



Scalability

Flexible and modular to scale with your testing needs.

TRANSFORMING COMPLEXITY INTO CLARITY. DELIVERING IMPACT.



Current Challenges in NGS Implementation

From sample preparation and library construction to enrichment, analysis, and quality control, conventional NGS workflows often remain labor-intensive and operationally complex.



1. Labor-Intensive Workflow

Manual handling across multiple steps increases staff burden and hands-on time.



2. Technical Complexity

Library prep, enrichment, QC, and data workflows require specialized training and experience.



3. Turnaround Time Pressure

Extended manual processes can lengthen TAT and limit testing capacity.



4. Quality Consistency Risk

Operator-to-operator and batch-to-batch variation can affect result stability.



5. Cost and Resource Burden

Rework, failed runs, and high manpower requirements increase operational cost.



6. Scalability Challenges

Complex workflows are difficult to replicate across programs, sites, or growing test volumes.

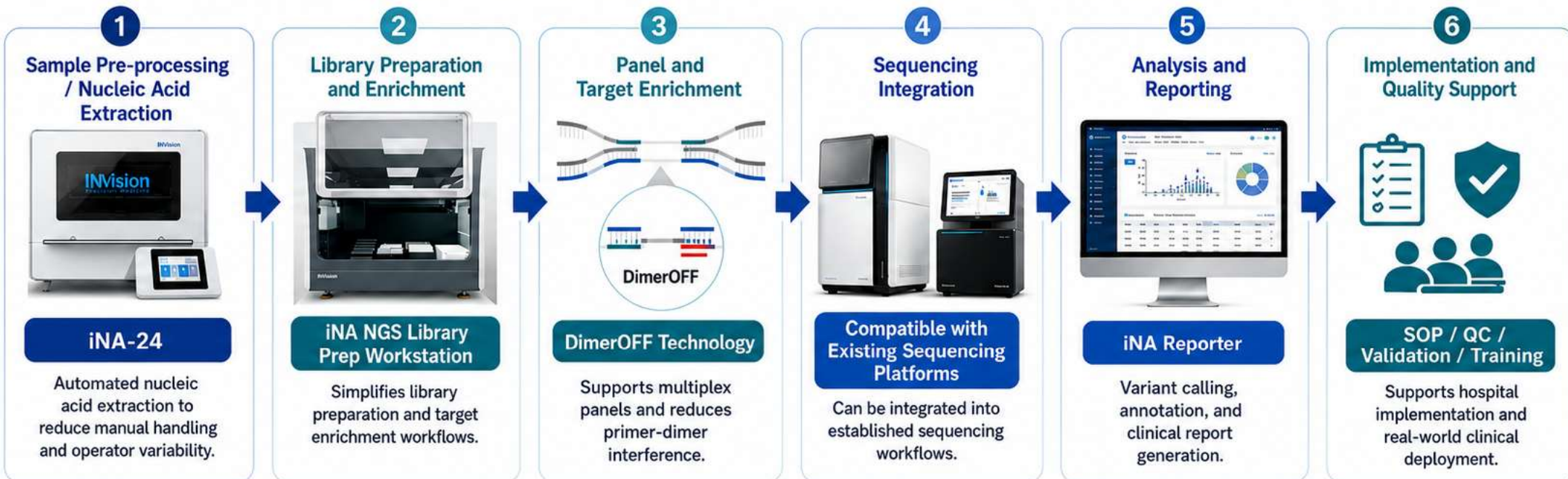


Core challenge: conventional NGS implementation often faces high labor dependency, longer TAT, quality variability, and limited scalability.

INVision Sample-to-Report Integrated Workflow

Data IN,
Report OUT

INVision transforms complex NGS testing into a standardized, automated, and deployable in-house solution.



Automation

Automated instruments and workflows improve efficiency and reduce manual workload.



Standardization

Standardized SOP and QC framework help ensure consistent and reproducible results.



Deployability

Designed for in-house hospital implementation, enabling rapid adoption and practical deployment.



Scalability

Modular design with flexible expansion to support broader testing needs.



Starting with Leukemia,
expandable to TP53, RNA Fusion,
FFPE, Pan-Cancer, and cfDNA.



Leukemia



TP53



RNA Fusion



FFPE



Pan-Cancer



cfDNA

From Central Lab to Market Expansion



Scalable. Standardized. Sustainable.



One Central Lab. Two Strategic Pathways. Limitless Impact.

INLab NGS Precision Medicine Solution

Scalable Manufacturing & OEM/ODM Capabilities



Class 100,000 Cleanroom

Meets stringent particulate and contamination control standards



Automated Manufacturing

Supports scalable production of precision medicine instruments and nucleic acid extraction/purification reagents



Maximum Annual Capacity per Line

250,000
tests/year/line

Expandable to multiple lines based on demand



OEM/ODM Services

Contract manufacturing and turnkey production-line deployment

Designed and Manufactured in Taiwan

SOLID TUMOR & LIQUID BIOPSY PANELS



Liquid Biopsy

cfDNA · 4 genes



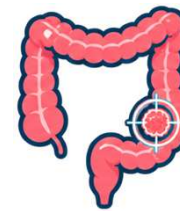
BRCA1/2

2 full genes



Lung Cancer

12 genes



Colon Cancer

14 genes



Breast Cancer

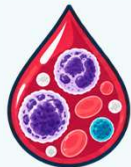
12 genes



Pan-Cancer

27 genes

HEMATOLOGIC MALIGNANCY PANELS



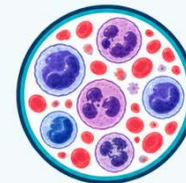
MPN

5 genes



TP53

Full CDS



Leukemia

35 genes

RNA FUSION PANEL



RNA Fusion

105 genes

Solid & hematologic tumors



Pan-Cancer Hotspots V1

27 genes · 80 exons

COVERED GENES

AKT1 · ALK · APC · BRAF
CCND1 · CTNNB1 · EGFR
ERBB2 · ERBB3 · ESR1 · FBXW7
FGFR1 · GNAS · IDH1 · IDH2
JAK2 · KRAS · MAP2K1 · MET
NRAS · NPM1 · PIK3CA · RET
ROS1 · SF3B1 · SMAD4 · TP53

Broad hotspot coverage

Input	5–100 ng
Reactions	24 / 48 / 96
Throughput	>90 M bp
Coverage	≥3,000×
On-target	>90%

gDNA | cfDNA



Lung Cancer NGS Panel

12 genes · 41 exons

COVERED GENES

ALK · BRAF · EGFR · ERBB2
KRAS · MAP2K1 · MET · NRAS
PIK3CA · RET · ROS1 · TP53

Actionable driver coverage

Input	5–100 ng
Reactions	48 / 96
Throughput	>50 M bp
Coverage	≥3,000×
On-target	>90%

gDNA | cfDNA



BRCA NGS Panel

2 full genes · 51 exons

COVERED GENES

BRCA1 · BRCA2

Full coding sequence (CDS)

Comprehensive BRCA coverage

Input	2.5–100 ng
Reactions	24 / 48 / 96
Throughput	>50 M bp
Coverage	≥1,000×
On-target	>90%

gDNA | cfDNA



cfDNA NGS Panel

4 genes · 11 exons

COVERED GENES

BRAF · EGFR
KRAS · NRAS

Focused liquid-biopsy panel

Input	5–100 ng
Reactions	48 / 96
Throughput	>17 M bp
Coverage	≥3,000×
On-target	>95%

gDNA | cfDNA

COMMON WORKFLOW <200 bp amplicons · Paired-end 150 bp · Cell line / blood / bone marrow / FFPE · cfDNA panel also supports urine



RNA Fusion Panel Plus

105 genes

>100 fusion events
85 solid-tumor fusions
19 hematologic fusions

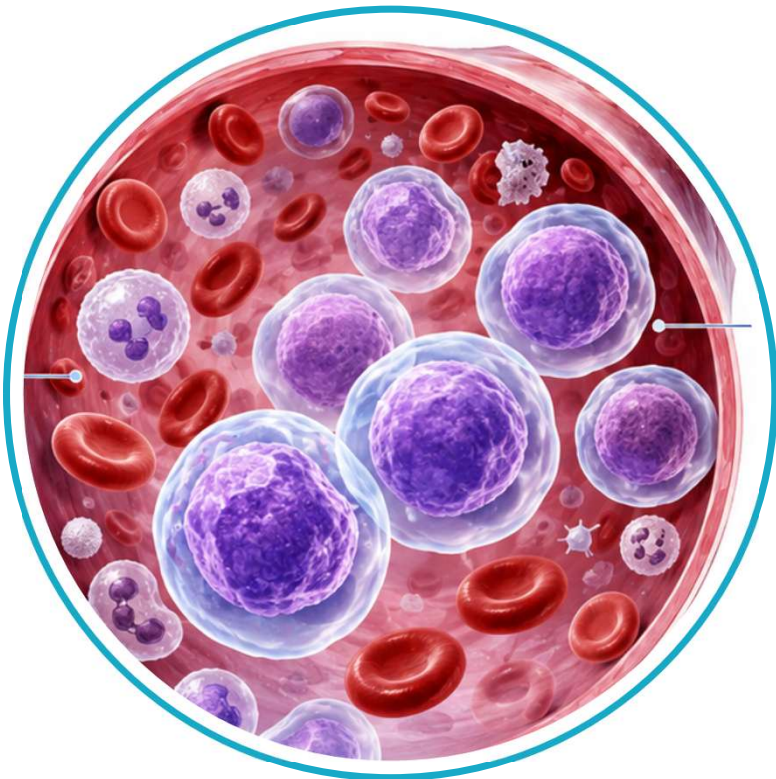
REPRESENTATIVE ACTIONABLE FAMILIES

ALK · RET · ROS1
NTRK1 / NTRK2 / NTRK3
FGFR1 / FGFR2 / FGFR3 · MET

RNA input 20–100 ng
 24 / 48 / 96 reactions
 ≥3,000× mean coverage · >90% on-target
 Recommended throughput >50 M bp

Cancer type	NHI-listed biomarkers	iNA panel coverage
Non-Small Cell Lung Cancer 9 listed categories	MUTATIONS EGFR · MET exon 14 skipping BRAF · ERBB2 · KRAS FUSIONS ALK · NTRK · ROS1 · RET	DNA PANELS EGFR · MET · BRAF · ERBB2 · KRAS RNA FUSION PANEL ALK · NTRK1/2/3 · ROS1 · RET ✓ All 9 listed categories represented
Triple-Negative Breast Cancer	Germline BRCA1 & BRCA2 comprehensive analysis	BRCA Panel BRCA1 + BRCA2 full CDS ✓
Ovarian / Fallopian Tube / Primary Peritoneal Cancer	Germline or somatic BRCA1 & BRCA2 comprehensive analysis	BRCA Panel BRCA1 + BRCA2 full CDS ✓

NHI mapping reproduced from the supplied internal reference; confirm the latest reimbursement criteria before external or clinical use.



Hematopoietic stem cell → leukemic blast expansion

Mutation · Fusion · Molecular follow-up

AML COVERAGE ACROSS DNA + RNA

21 mutation genes + 4 key fusions



iNA Leukemia NGS Panel

35-gene DNA panel

NHI-LISTED MUTATION GENES COVERED (21)

FLT3 · ASXL1 · CEBPA · DNMT3A
IDH1 · IDH2 · JAK2 · KIT · KRAS
NPM1 · NRAS · PTPN11 · RUNX1
SETBP1 · SF3B1 · SRSF2 · TET2
TP53 · U2AF1 · WT1 · ZRSR2

ADDITIONAL MYELOID GENES INCLUDE

BCOR · CALR · CBL · CSF3R · DDX41
ETV6 · EZH2 · GATA2 · MPL · PHF6
PIGA · PPM1D · STAG2

iNA RNA Fusion NGS Panel

19 hematologic fusions

NHI-LISTED FUSIONS COVERED (4)

BCR::ABL1 · PML::RARA
CBFB::MYH11 · RUNX1::RUNX1T1

ADDITIONAL FUSIONS

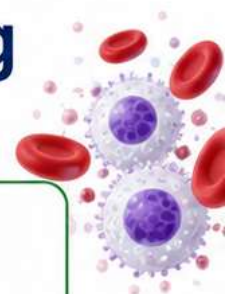
DEK::NUP214 · KMT2A::ENL / ELL
PAX5::JAK2 · KMT2A::AF9 / AF10
RBM15::MKL1 · TCF3::PBX1
NUP98::HOXA9 / NSD1 · KMT2A-PTD
CIC::DUX4 · KMT2A::AF4 / AFDN
ETV6::RUNX1

Together, the DNA + RNA panels represent all 25 AML alterations listed in the supplied NHI mapping.

Mapping is based on the supplied reference and should be reconfirmed against current reimbursement criteria before external use.

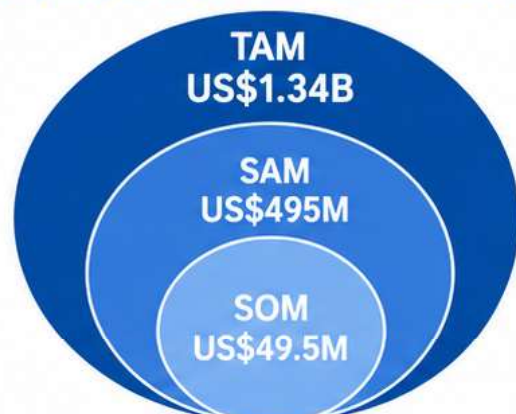
Market Size: Hematologic Malignancy Molecular Testing

Global opportunity for clinical-decision testing and molecular follow-up



Clinical Decision Testing

Newly diagnosed patients (global annual incidence)



- **TAM:** 1,338,558 new hematologic malignancy cases/year × US\$1,000/test
- **SAM:** Leukemia-only core focus = 495,113 new cases/year × US\$1,000/test
- **SOM:** 10% of SAM

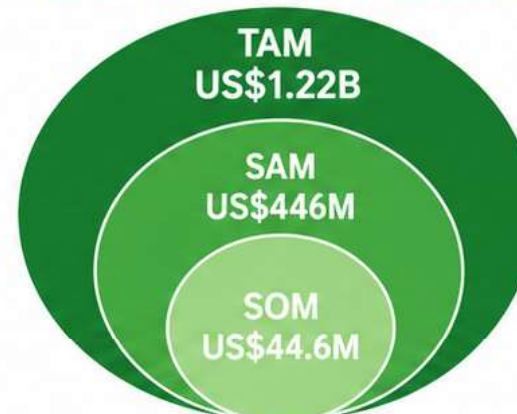


Price assumption: US\$1,000 per clinical-decision test



Molecular Follow-up

Global patients in follow-up (5-year prevalence; 1 test/year)



- **TAM:** 4,059,324 hematologic malignancy patients in 5-year prevalence × US\$300/test/year
- **SAM:** Leukemia-only follow-up = 1,488,016 patients × US\$300/test/year
- **SOM:** 10% of SAM



Price assumption: US\$300 per follow-up test



Base-case Total TAM: US\$2.56B/year

Clinical decision testing US\$1.34B/year + molecular follow-up US\$1.22B/year



TAM includes leukemia, non-Hodgkin lymphoma, Hodgkin lymphoma, and multiple myeloma.

SAM is shown as a conservative leukemia-only addressable market based on current panel focus.

SOM assumes capture of 10% of SAM.



Clinical Validation Partner: Yale School of Medicine



Real-world clinical validation of INB's leukemia precision medicine solution by international hematopathology experts

Why Yale Validation Matters

INB does not rely solely on internal R&D data. The **iNA Leukemia NGS Panel** is being evaluated at a leading U.S. medical center using real-world leukemia specimens and benchmarked against established clinical NGS results.

Clinical Validation Design

- Institution: Yale School of Medicine / Yale New Haven Hospital
- Principal Investigator: Prof. Henry Rinder
- Setting: Clinical hematopathology and molecular diagnostics laboratories
- Evaluation: iNA Leukemia NGS Panel and automated precision medicine workflow
- Objectives: Pathogenic-variant detection, VAF concordance, and negative-sample classification

Clinical Significance

- Uses real-world leukemia specimens, beyond R&D samples
- Demonstrates detection of clinically significant variants
- Supports hospital adoption, multinational validation, and commercial collaboration
- Reduces operator variability and improves laboratory standardization



Clinical Validation Partner: Yale School of Medicine (USA)



Yale University
School of Medicine



Yale School of Medicine

- ◆ Professor of Laboratory Medicine and of Medicine (Hematology)
- ◆ Vice Chair for Clinical Affairs, Laboratory Medicine;
- ◆ Associate Director, Yale Pathology Residency Program;
- ◆ Director, Clinical Hematology Laboratory

Professor Henry Rinder

- Yale School of Medicine
- American Society for Clinical Pathology
- American Society for Clinical Pathology (ASCP)

◆ President of the ASCP Commission on Continuing Professional Development



INLab Automated Precision Medicine Solution



Partnership Model: Yale provides the clinical setting and reference data; INB provides the automated leukemia NGS panel and precision medicine workflow. Together, they evaluate platform performance using real-world clinical samples.

Clinical Validation: iNA Leukemia NGS Panel

Real-world clinical leukemia specimens demonstrate high detection performance and strong concordance with Yale results



Yale School of Medicine / Yale New Haven Hospital

Validation Objective

Using Yale's established NGS results as the reference, evaluate the iNA Leukemia NGS Panel's pathogenic variant detection rate, VAF concordance, and negative-sample performance in real-world clinical specimens to support clinical implementation and international market expansion.

Clinical Validation Results

91.86%

Pathogenic Variant Detection Rate

158

Concordant Pathogenic Variants
Tier 1 & Tier 2 variants

41

Non-Pathogenic Variants
Detection Rate: 91.11%

24

Negative Samples
Negative Agreement: 91.67%

N = 96

Real-world clinical leukemia specimens

Strong Correlation Between iNA and Yale Results



158
Level 1 & 2 Mutations

VAF Concordance Assessment

- Yale-measured VAF values were compared with iNA platform VAF values
- 158 Level 1 & 2 mutations demonstrated a strong concordance trend
- Supports variant detection using the iNA Leukemia Panel in real-world samples

Validation Design & Clinical Significance

Real-World
Leukemia Specimens

iNA Panel
Testing

Comparison with
Yale Results

Concordance
Assessment

Validation Design

- Method comparison using real-world clinical samples
- Comparison of iNA Panel results with Yale's established NGS results
- Assessment of pathogenic-variant detection and negative-sample performance
- Strengthens confidence for clinical implementation and international collaboration

Clinical Value

✓ **High Sensitivity**

Detects clinically significant variants

✓ **High Accuracy**

Strong agreement with medical center results

✓ **Greater Efficiency**

Automation reduces operator variability

✓ **International Validation**

Builds global market confidence

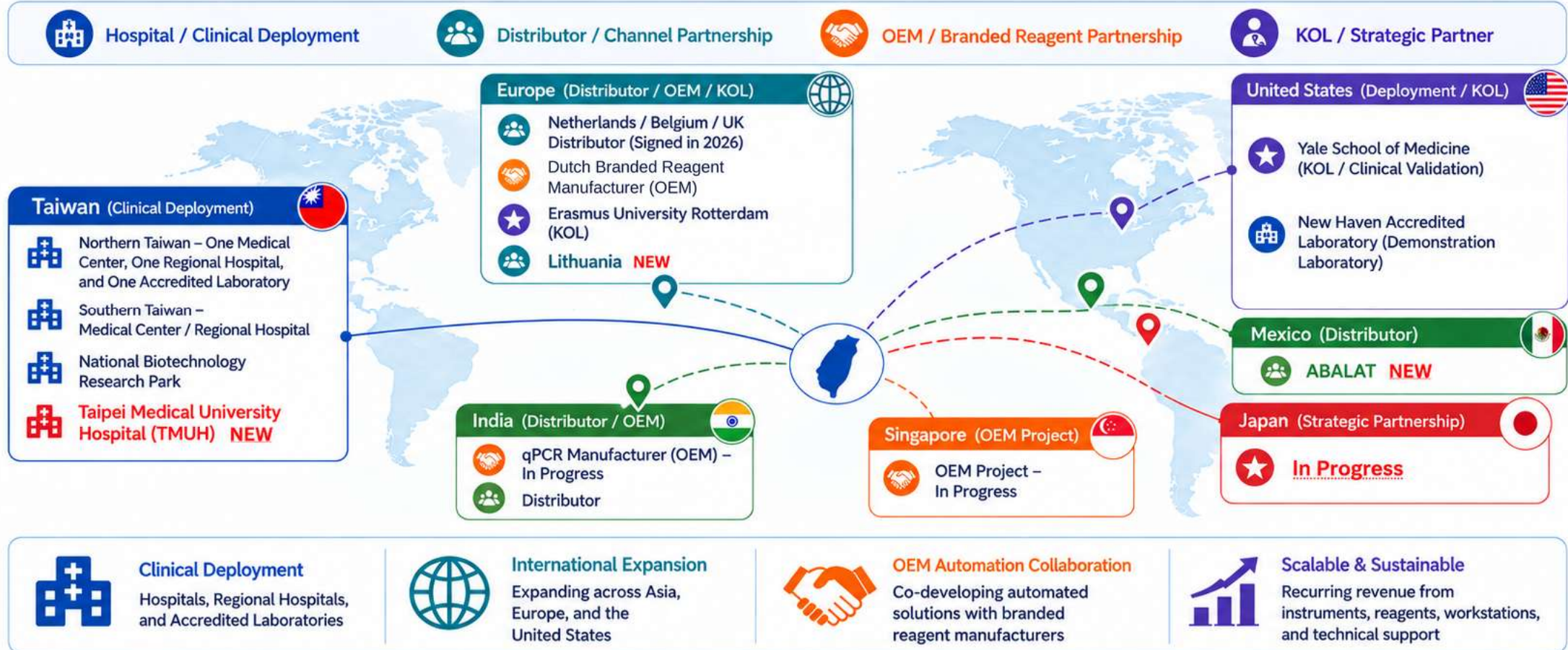
Across **96** real-world clinical leukemia specimens, the iNA Leukemia NGS Panel achieved a **91.86%** pathogenic variant detection rate and demonstrated strong concordance with Yale sequencing results—supporting larger-scale clinical validation, hospital implementation, and international market expansion.

iNA | INLab Precision Medicine Solution

Global Footprint & Deployment Progress



Starting with clinical deployment, we connect channel partners and OEM automation partners to expand our global footprint.



INVISION is committed to becoming a key global partner in precision medicine automation—advancing NGS from manual to automated, and from local to global.

PROTEIN DIVISION



THE LIGHT SAVES LIVES

Light-Based Technology for Life-Saving Impact



ULTRA-HIGH SENSITIVITY

Detects low-abundance protein signals



REAL-TIME ANALYSIS

Label-free binding kinetics in real time



RELIABLE & REPRODUCIBLE

Consistent data for clinical translation



PROTEIN CHARACTERIZATION

Kinetics | Affinity | Epitope



ANTIBODY DEVELOPMENT

Screening | Selection | Pairing



BIOMARKER ANALYSIS

Label-Free | Ultra-Sensitive



TRANSLATIONAL & CLINICAL USE

Research to Real-World

INNOVATION | PRECISION | RELIABILITY

Transforming Healthcare with Light





Unlocking the Hidden Potential of Antibody Libraries

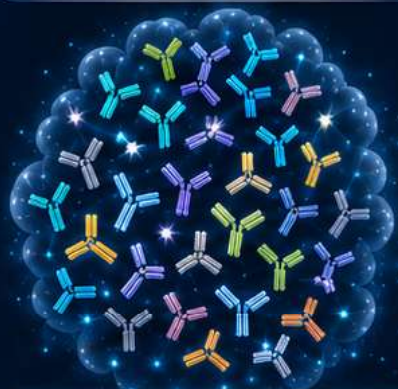
From FOPPR Characterization to
FONLISA Ultra-Sensitive Detection



Identify **High-Value, High-Specificity, and High-Sensitivity** Antibodies from an Existing Monoclonal Antibody Pool

Accelerate screening and support applications in diagnostic immunoassays, antibody drugs, and precision medicine

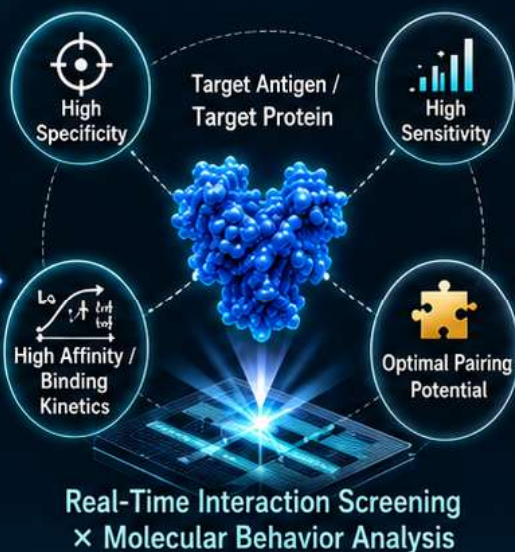
1 Existing monoclonal antibody pool



Price-competitive and difficult to differentiate, yet still an underdeveloped treasure trove

Large number of existing monoclonal antibody candidates

2 Selection of high-value antibodies



Precisely Select High-Value Antibodies

3 High-value antibody output



4 Downstream applications



Diagnostic Immunoassay

Improve detection sensitivity and accuracy



Antibody Drug

Support candidate antibody evaluation and development



Precision Medicine

Advance biomarker development and clinical translation



Shorten Screening Time



Reduce Trial-and-Error Costs



Accelerate R&D and Translational Applications



FOPPR Platform Applications

Real-Time, Label-Free 3D Sensing for Antibody & Biomarker Development

1 Research & Laboratories

Antibody screening; affinity / kinetics analysis; epitope mapping

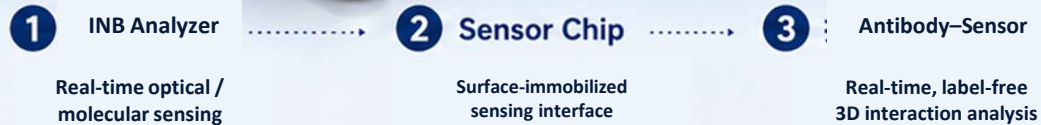


2 Biopharma & Manufacturing

High-value antibody development; multiplex analysis; blocking, competition, and co-binding



Powered by **FOPPR®**



3 CROs & Bioanalytical Services

Method and potency development; project evaluation; reproducibility and technical validation



4 IVD & Diagnostic Development

Antibody pairing; enhanced sensitivity; biomarker development; clinical translation



Real-Time Analysis

Instant kinetic data acquisition



Label-Free

No fluorescent labels or labeled antibodies required



High Sensitivity

Detects subtle molecular changes



Multi-Antibody Interaction Analysis

Competition, blocking, and co-binding in a single run

Light Sensing Biomarker Analyzer : A Multiplex Application Platform

After obtaining IVDR certification, the platform can be extended to multiple disease applications.
Alzheimer's disease is one key application.



One Platform, Multiple Assays

Shared technology and workflow to improve R&D efficiency



One Platform, Multiple Diseases

Rapidly extend to various disease areas for broader impact



From Product to Application Platform

Create long-term value and drive sustained market growth



WHY NOW?

MARKET POTENTIAL OF ALZHEIMER'S BLOOD TESTING

Population aging, a large unmet need, maturing blood biomarkers, and decentralized testing are accelerating market growth.

1 LARGE UNMET NEED



55+ MILLION
people worldwide live
with dementia

Alzheimer's disease is the leading cause of dementia, driving growing demand for screening and follow-up.

2 AGING DRIVES TESTING DEMAND



BY 2030
1 IN 6 PEOPLE
worldwide will be
aged 60 or older

Population aging will increase demand for risk assessment, early detection, and long-term monitoring.

3 BLOOD BIOMARKERS ENTER CLINICAL PRACTICE



Alzheimer's blood biomarkers are rapidly moving into clinical use

Compared with imaging and invasive tests, blood testing is more accessible, scalable, and commercially promising.

4 DECENTRALIZED TESTING OPENS NEW MARKETS



US\$9 BILLION
potential market opportunity

Deployment across communities, health-screening centers, and primary care can expand coverage and testing penetration.

ALZHEIMER'S BLOOD TESTING ACROSS THE CONTINUUM OF CARE



RISK ASSESSMENT

Identify high-risk populations and establish baseline data



EARLY DETECTION

Detect abnormalities before clinical symptoms emerge



PRE-TREATMENT ASSESSMENT

Assess pathology status and treatment suitability



POST-TREATMENT MONITORING

Monitor treatment response and disease progression



INVESTMENT HIGHLIGHT

Alzheimer's blood testing is more than a single diagnostic product. It can expand into large-scale screening, companion diagnostics, clinical-trial recruitment, and long-term monitoring—creating a scalable growth market.

SCIENTIFIC ADVISOR



Professor Charlotte Teunissen

World-Renowned Alzheimer's Disease Research Scientist Professor Charlotte Teunissen

- ✓ **Co-Chair, Alzheimer's Association Global Blood-Based Biomarker Standardization Consortium**

Advancing biomarker standardization for global clinical practice

- ✓ **Member, WHO Expert Advisory Group on Blood-Based Biomarkers for Alzheimer's Disease**

- ✓ **Professor of Neurochemistry and Head of the Neurochemistry Laboratory, Amsterdam UMC**



Amsterdam UMC

University Medical Centers

Disclosures: Collaboration contracts with ADx Neurosciences, Quanterix and Eli Lilly; contract research or grants from AC Immune, Axon Neurosciences, BioConnect, Biorchestra, Brainstorm Therapeutics, Celgene, EIP Pharma, Eisai, Fujirebio, Grifols, **Instant Nano Biosensors**, Merck, Novo Nordisk, PeopleBio, Roche, Siemens, Toyama and Vivoryon; prior speaker contracts with Roche, Grifols and Novo Nordisk.



INTERNATIONAL SCIENTIFIC FORUM PRESENTATION



AD/PD™ 2024 ADVANCES IN SCIENCE & THERAPY

International Conference on
Alzheimer's and Parkinson's Diseases
and related neurological disorders

Unlocking Low-Abundance Biomarkers for Precision Medicine



Tunable Ultra-sensitivity
at Femtomolar Detection



Ultra-Specificity
Detection Signal Level



Broad Applications &
Accelerated Translation

1. Affinity Analysis



FOPPR® Platform

2. Epitope Binning



FOPPR® Platform

3. Ultra-Sensitive Detection



FOPPR® Platform

Development of a prototype point of care test for detection of neurofilament light chain in plasma

Madison I. J. Honey, MSc,¹ Andrew Chang, PhD², Daniel
Antwi-Berko, PhD¹, Tony Chung², Inge M. W. Verberk,
PhD¹, Wiesje M. van der Flier, PhD³, Charlotte E.
Teunissen, PhD¹

¹ Amsterdam UMC, The Netherlands

² Instant NanoBiosensors, Taiwan



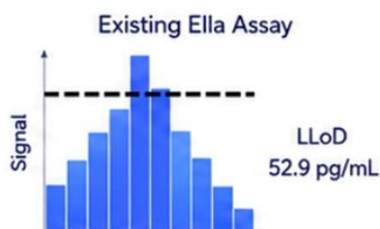
Breakthrough Technology Presentation



From Undetectable Samples to an 86× Lower LLoD

FOPPR-guided antibody selection improved downstream assay sensitivity

1 Challenge



? The existing Ella assay had an LLoD of 52.9 pg/mL, leaving multiple samples undetectable.

It also relied on polyclonal antibodies, increasing the risk of batch variability and cross-reactivity.

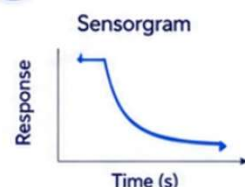


Batch variability

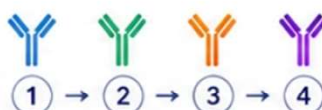


Cross-reactivity

2 FOPPR-Guided Antibody Selection



- Real-time kinetic analysis



- Sequential epitope binning



Antibodies showing greater response to a homologous non-target protein than to the target protein were excluded.

3 Outcome

FOPPR-Selected Antibody Pair



Transferred to Simoa



52.9 pg/mL → 0.618 pg/mL

≈ 86× lower LLoD

The selected pair was transferred to Simoa. Following platform-specific assay optimization, the reported LLoD improved to 0.618 pg/mL.



Problem:
Undetectable samples



FOPPR:
Better antibody selection



Result:
Higher sensitivity on Simoa

ALZpath Collaboration Ecosystem & Impact

Advancing Early Alzheimer's Diagnosis Through the pTau217 Blood Biomarker



TIME

Best Inventions 2024

ALZpath Named a 2024 Major Innovation



A. Licensing / Diagnostic Platforms

Abbott

Roche

**SIEMENS
Healthineers**

**BECKMAN
COULTER
Diagnostics**



ALZpath

Pathways to a better life



**ALZpath
proprietary
pTau217 antibody**

High sensitivity • Scalable • Non-invasive

Core technology for
blood-based testing



B. Research / Ultra-Sensitive Platforms

Quanterix®

**ALAMAR
BIOSCIENCES**

bio-techne®



C. Clinical Laboratory Collaboration



NEUROCODE



Joint Scientific Collaboration

Tau 2024 Co-presented Poster

Building collaboration visibility through
Tau / Alzheimer's biomarker research

ALZpath × INB

**Collaboration
Milestones**



2024

Roche / Beckman



2025

Siemens



2026

Abbott

INB Global Footprint & Partnership Progress

Global deployment, distributors, strategic collaborations, and validation/service presence.

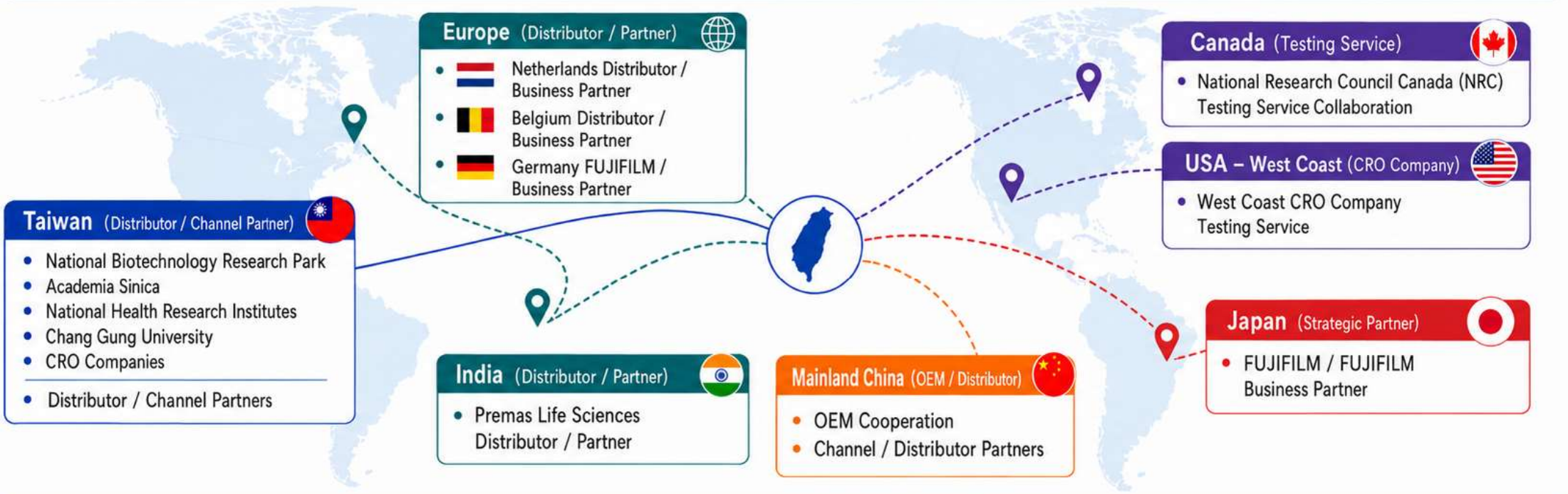


Clinical / Service Sites

Distributors / Channel Partners

Brand Partners

Strategic / KOL Collaborations



Clinical / Service Deployment
Testing services and validation partnerships worldwide

Global Expansion
Strengthening presence across Asia, Europe, and North America

Strategic Brand Partnerships
FUJIFILM Japan and FUJIFILM Germany partnerships

Scalable, Recurring Business
Building sustainable, high-margin, recurring revenue streams

INB continues to expand from Taiwan to Asia, Europe, and North America, advancing research applications, commercial partnerships, and global market expansion.



Unlocking the Hidden Potential of Antibody Libraries

From FOPPR Characterization to
FONLISA Ultra-Sensitive Detection



TAU>2024

Global Conference

ALZHEIMER'S
ASSOCIATION

curePSP
UNLOCKING THE SECRETS OF BRAIN DISEASE

RAINWATER
Charitable Foundation

pTau217 Assay Development

Instant NanoBiosensors Co., Ltd.

INSTANT NanoBiosensors ALZpath Rapid Analysis and Validation of Antibody Pairs in a Few Days' Journey: Developing a pTau-217 Assay Using Fiber Optic Particle Plasmon Resonance (FOPPR) Technology

Ting-Chau A. Chang, PhD¹, Feng-Yi Ke, PhD¹, Po-Jen Chien, PhD¹, Tsun-Chih Chung¹, Lauren Chaby, PhD², Andreas Jeromin, PhD²

¹Instant Nanobiosensors Co., Ltd., National Biotechnology Research Park, Taipei City, Taiwan

²ALZpath, Inc., Carlsbad, California, USA

Keywords: Fiber-Optic Particle Plasmon Resonance, FOPPR, p-Tau217, PTMs, FONLISA

Theme: Biomarkers-Non-Neuroimaging Email: info@instantbiosensors.com

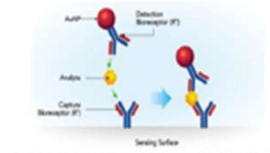
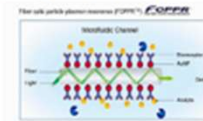


Background:

The development of a pTau-217 assay leveraging Fiber Optic Particle Plasmon Resonance (FOPPR) technology is designed to detect the abnormally phosphorylated tau protein variant, pTau-217, which is significant in neurodegenerative diseases like Alzheimer's. The goal is to establish pTau-217 as a reliable biomarker for point-of-care (POC) usage in both acute clinical settings and patient monitoring. This involves a meticulous process of identifying the most suitable pTau-217 antibody pairs through in-depth affinity and epitope binning analyses. FOPPR technology facilitates the effective analysis of antibodies that have undergone post-translational modifications (PTMs), aiding in the selection of the optimal antibody pair. Following this, the pTau-217 levels in EDTA plasma samples are quantified with enhanced precision and sensitivity utilizing the Fiber Optic Nanogold-linked Immunosorbent Assay (FONLISA) method.

Methods:

The FOPPR technology incorporates a carefully designed self-assembled monolayer (SAM) to mitigate retention effects and minimize rebinding, ensuring the accuracy of the measured affinity constants. Additionally, the system employs an auto-flowing microfluidic chip that mimics the velocity of blood through veins, simulating the physiological environment of the body. This approach allows for the evaluation of binding affinities under conditions that closely resemble real-world biological interactions. For the pTau-217 assay, nine antibodies were initially tested for their affinity for Tau protein, and two were excluded due to low affinity. The remaining antibodies were ranked by their affinity constants through epitope binning to identify their binding sites on Tau N-terminal or pTau-217. In FOPPR technology, the optical fiber set in a microfluidic chip detects nanoplasmic absorption changes caused by the formation of the immunocomplex, using the INB D200 device. The assay's sensitivity (LOD) was ascertained through blank measurements, with its linear range established using a pTau-217 calibrator.



Results:

The affinity constants for the antibodies ranged from a high of 0.4 nM to a low of 655 nM, with one antibody not yielding results. Epitope binning identified that antibodies A2, I2 (polyclonal), and ALZpath detection antibody, as well as B1 and A2-1, recognized the same epitope of the pTau-217 calibrator while antibody I1 (polyclonal) bound to a different epitope. The monoclonal ALZpath detection antibody and ALZpath capture antibody demonstrated the strongest affinity for the pTau-217 calibrator. For the assay, the ALZpath detection antibody was immobilized on the optical fiber core, and the ALZpath capture antibody was attached to gold nanoparticles. This setup provided a linear detection range from 0.0024 pg/mL to 10 pg/mL for pTau-217, with the lower limit of detection (LOD) being less than 2.4 fg/mL. Recovery and CV in a duplicated spike test of the pTau-217 calibrator showed 96.8% recovery with a CV of 0.6% at low concentration (0.096 pg/mL), and a recovery of 101.8% with a CV of 2.7% at a higher concentration.

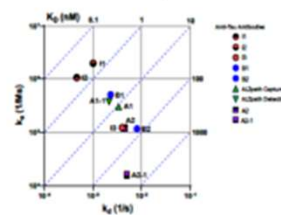


Figure 1. The affinity distribution of nine antibodies. The antibodies ALZ capture antibody A2, and I2 recognize pTau-217, while the rest recognize the N-terminal of Tau.

Table 1. Quantitative measurement of antibody affinity

Antibody	IS	I2	B1	B2	ALZpath capture	A2	A2-1
K_d (nM)	3.91×10^{-5}	1.75×10^{-5}	2.35×10^{-5}	2.35×10^{-5}	2.35×10^{-5}	2.35×10^{-5}	1.42×10^{-5}
LOD (nM)	2.35×10^{-5}	2.35×10^{-5}	2.35×10^{-5}	2.35×10^{-5}	2.35×10^{-5}	2.35×10^{-5}	2.35×10^{-5}
LOD (pg/mL)	0.01	0.01	0.01	0.01	0.01	0.01	0.01

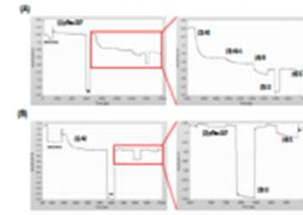


Figure 2. The sensorgram of epitope binning. (A) The tandem format and (B) Sandwich format of epitope binning analysis. TDX-NP to active carboxylic acid for immobilize protein on optic fiber. Ethylenediamine (EDA) add to interactive with excess carboxylic acid.

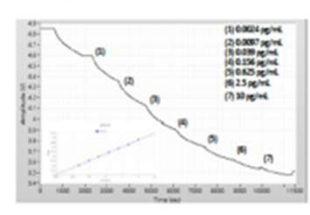


Figure 3. Real-time detection with ALZpath antibody pair for calibration curve upon injection of different pTau-217 calibrator concentrations ranging from 0.0024 pg/mL to 10 pg/mL (74 series).

Table 2. Spiked Test with ALZpath antibody pair on FOPPR technology

Prepared Conc. (pg/mL)	Average Conc. (pg/mL)	Recovery (%)	CV (%)
0.096	0.093	96.8%	0.6%
0.56	0.57	101.8%	2.7%

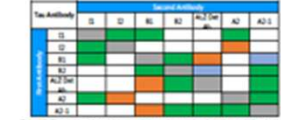


Figure 3. The epitope binning map of these four antibodies. I2 and ALZpath capture antibody did not undergo epitope binning due to material issues.

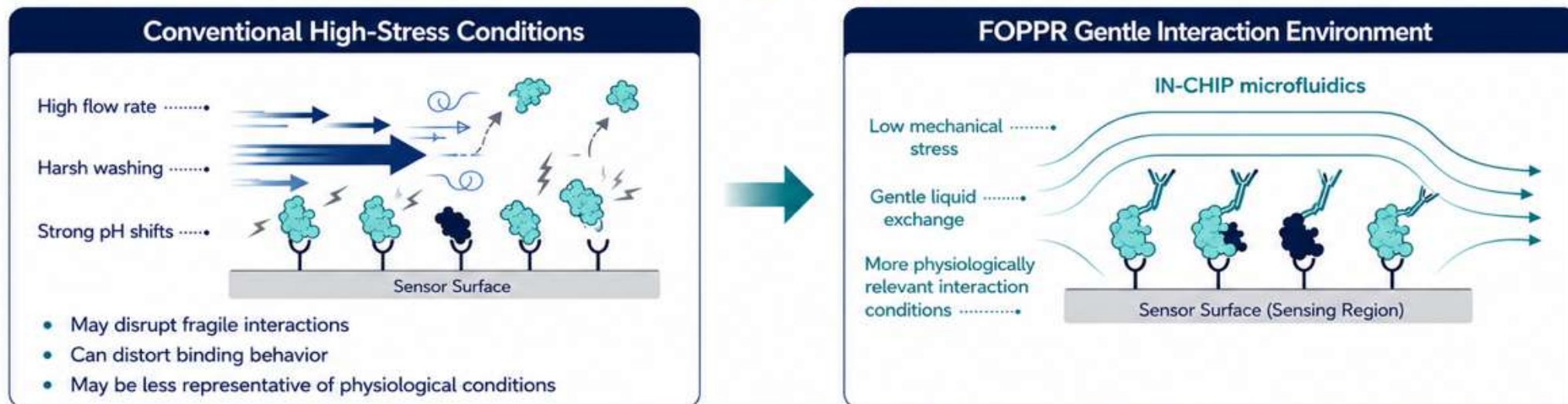
Conclusion:

The assay, based on FOPPR technology, effectively selected appropriate antibody pairs and demonstrated ultra-sensitive detection of pTau-217 in EDTA plasma. From initial antibody analysis to the validation of the antibody pair performance, preliminary results were typically obtained within a few days. The assay showed high specificity for pTau-217, with minimal cross-reactivity with other tau isoforms. Compared to existing methods, this assay offers a more sensitive and rapid detection of pTau-217, highlighting its potential for early diagnosis and monitoring of neurodegenerative diseases. The strong potential for POC applications is supported by continuous improvements aimed at enhancing technical stability and analytical sensitivity. Future work will focus on further validation of the assay in larger clinical cohorts and exploring its utility in monitoring disease progression and treatment response.

FOPPR Characterization

Gentle Interaction Under Near-Physiological Conditions

How IN-CHIP microfluidics supports gentle interaction characterization



Customer Value



Preserves fragile protein interactions



Supports comparative binding studies when controls are available



Supports real-time k_a , k_d , and K_D analysis under gentle conditions



Better foundation for downstream assay development



Intact Proteins



Selected Modified Protein Formats



Antibody Characterization



Assay Development

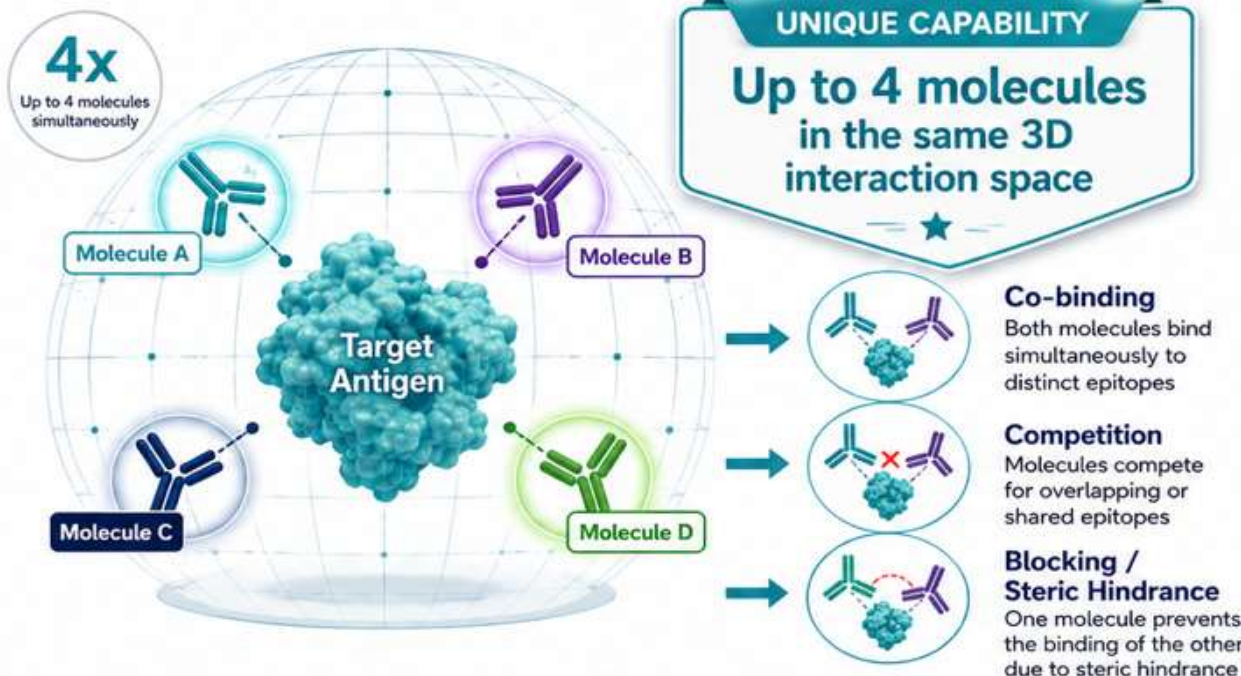
FOPPR Characterization

Unique 3D Interaction Space for Multi-Molecule Analysis

A powerful advantage for epitope binning, competition analysis, pair selection, and unique up-to-4-molecule analysis

Why 3D Space Matters

- More realistic interaction geometry
- Observe how multiple binders behave around the same target
- Better insight than simple one-to-one endpoint assays
- Applicable to intact proteins and selected modified protein formats



What FOPPR Can Reveal

- ★ **Unique: Up to 4 molecules** analyzed in the same 3D space
- Epitope overlap vs non-overlap
- Compatible capture / detection antibody pairs
- Drug candidate comparison on the same target
- Data to support differentiation and patent positioning



- 1 Affinity & Kinetics**
Quantify binding strength and rates



- 2 Specificity Assessment**
Evaluate binding specificity with relevant panels



- 3 Epitope Binning**
Map overlap and orientations



- 4 Pair Selection**
Choose optimal antibody pairs



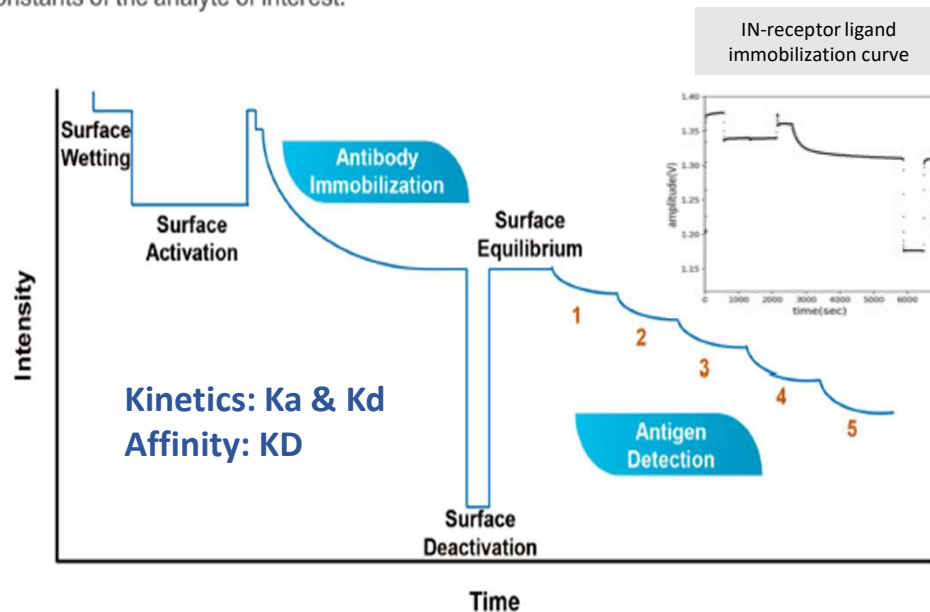
Unique 3D insight from up to four interacting molecules enables better assay decisions.

FOPPR Analysis: Kinetics & Affinity



Affinity Analysis Service

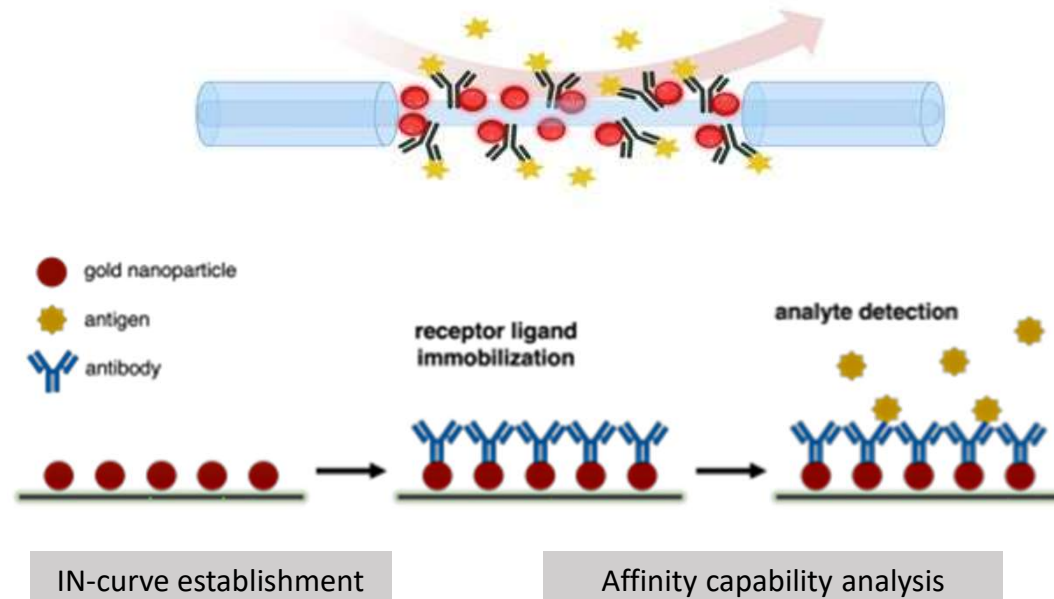
Antibody affinity is defined as strength of the binding interaction between antigen and antibody. It depends on the closeness of the stereochemical fit between antibody binding site and antigenic determinant, the size of the area of contact between them, and the distribution of charged and hydrophobic groups. The rate and strength of interactions between molecular species are important in biochemical research. These properties are measured through the kinetic and affinity constants of the analyte of interest.



Unique Features

Simulation of Real Cellular Molecular Reactions

- Cylindrical Sensor offers more structural space for molecular reactions
- IN-CHIP: gentle binding and dissociation conditions to avoid high-flow-rate and different PH environment

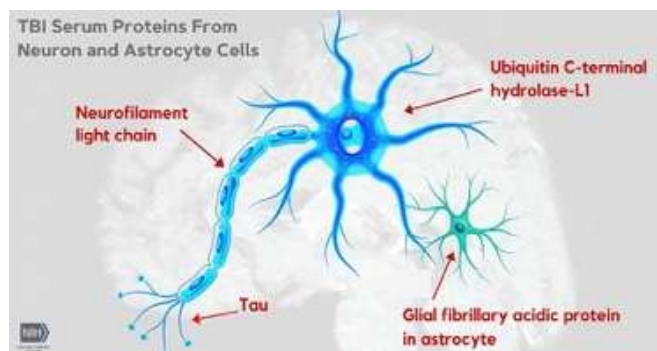


FOPPR Analysis : Kinetics & Affinity

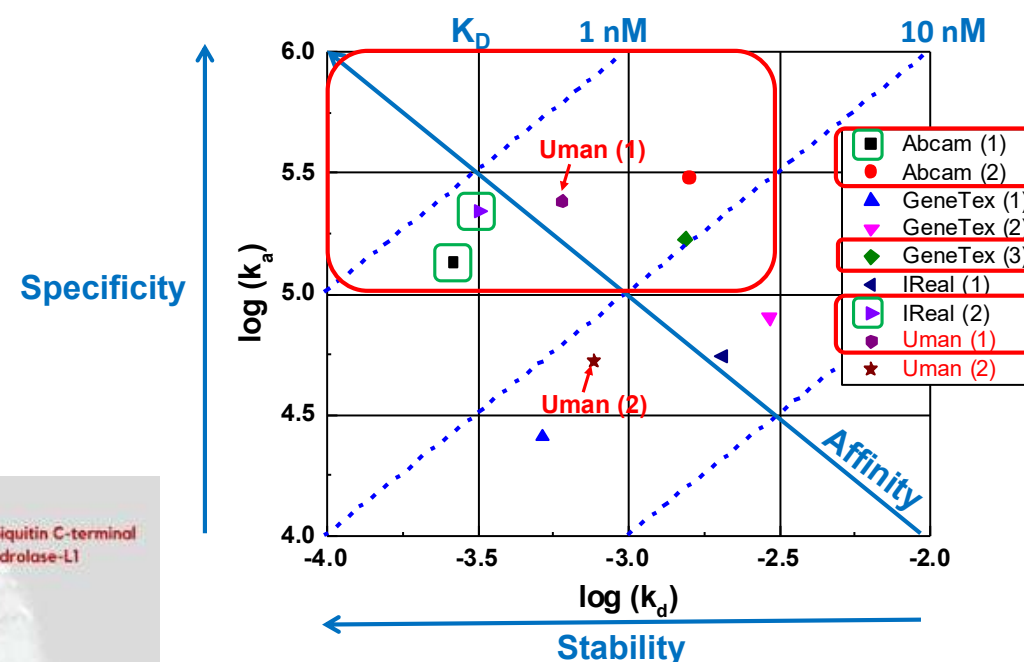
We are actively comparing the affinity of various branded antibodies in the market with the Uman antibody pair acquired by Quanterix, which has FDA certification. **Our aim is to identify a substitutable antibody pair with equivalent functionality, essential for future ultra-sensitivity detection tasks.**

It's noteworthy that in 2019, Quanterix acquired UmanDiagnostics, a leading supplier of Nf-L antibodies, for a sum of \$22.5 million USD.

Antibody Company	Clonal
Abcam (1)	Monoclonal
Abcam (2)	Monoclonal
GeneTex (1)	Monoclonal
GeneTex (2)	Monoclonal
GeneTex (3)	Polyclonal
IReal (1)	Polyclonal
IReal (2)	Polyclonal
Uman (1)	Monoclonal
Uman (2)	Monoclonal



Antigen	Antibody								
abcam	Abcam (1)	Abcam (2)	GeneTex (1)	GeneTex (2)	GeneTex (3)	IReal (1)	IReal (2)	Uman (1)	Uman (2)
k_a (1/MS)	1.35×10^5	3.01×10^5	2.57×10^4	7.98×10^4	1.68×10^5	5.54×10^4	2.20×10^5	2.42×10^5	5.28×10^4
k_d (1/s)	2.64×10^{-4}	1.60×10^{-3}	5.20×10^{-4}	2.93×10^{-3}	1.54×10^{-3}	2.06×10^{-3}	3.18×10^{-4}	6.01×10^{-4}	7.70×10^{-4}
K_D (nM)	1.96	5.34	20.26	36.79	9.19	37.24	1.44	2.48	14.58



Antigen Company	Sequence
Abcam	Full Length

Clinical Traction & Publications

NFL

A Fiber Optic Particle Plasmon Resonance Immunoassay for The Detection of Neurofilament Light Chain as An Early Biomarker for Diabetic Neuropathy

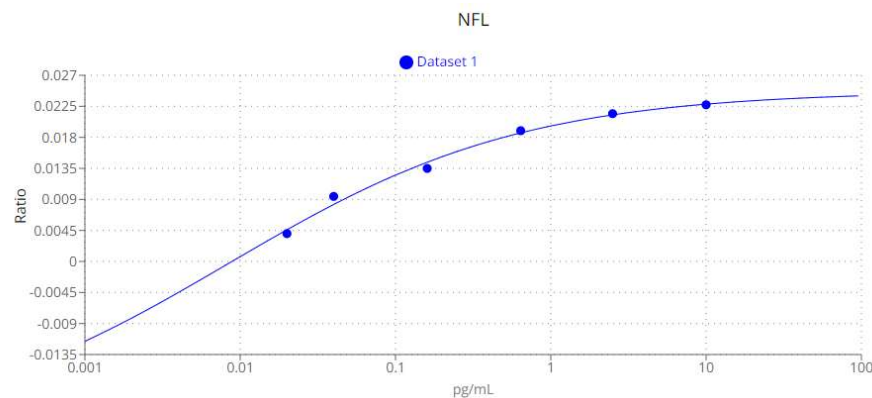
Fangjun Chen^{1,2,3}, Priscilla S.-W. Yeung^{1,2}, Ting-Chou Chang⁴, Tsun-Chih C
Hai-Wei Yen⁴, Run-Zhang Shi^{1,2}, Ruben Yiqi Luo^{1,2}

¹Department of Pathology, School of Medicine, Stanford University, Stanford, CA, USA

²Clinical Laboratories, Stanford Health Care, Palo Alto, CA, USA

³Department of Laboratory Medicine, Zhongshan Hospital, Fudan University, Shanghai, PR China

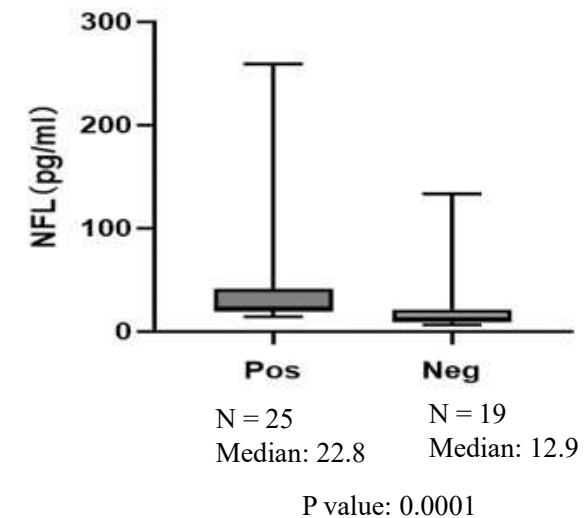
⁴Instant NanoBiosensors Co., Ltd.



Concentration Range: 0.02 pg/mL – 10 pg/mL

Plasma Dilution Factor: 20X

Plasma Sample Volume: 10 μ L



FOPPR Analysis: Epitope Binning



Epitope Binning Analysis

- **Accelerating Protein Drug Development**

Antibody Drug Candidate Analysis:

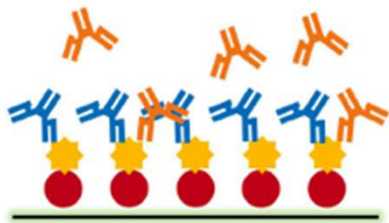
By sequentially interacting different antibody drugs with a single antigen in three-dimensional space, it's possible to analyze the antigen epitope relationships of up to four different candidate antibody drugs at the same time.

- **Accelerating IVD Development**

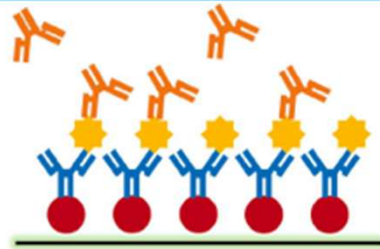
Antibody Pair Analysis:

Accurately select the optimal capture (Capture Ab) and detection (Detection Ab) antibody pair to reduce the time needed for reagent development.

Tandem Format

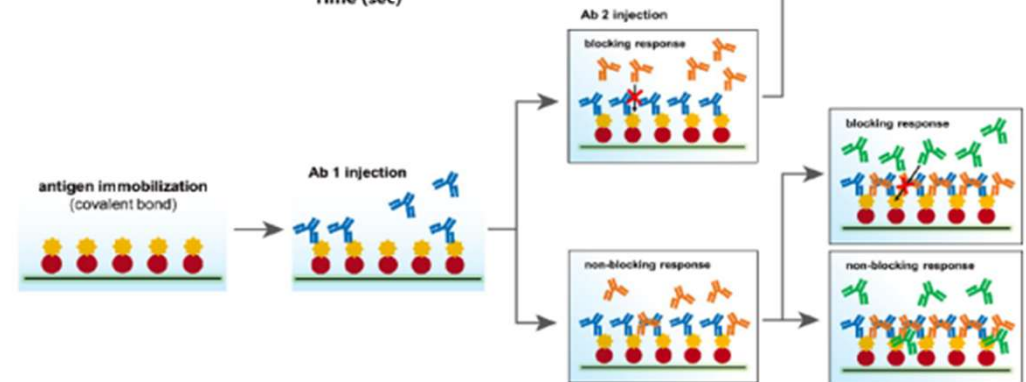
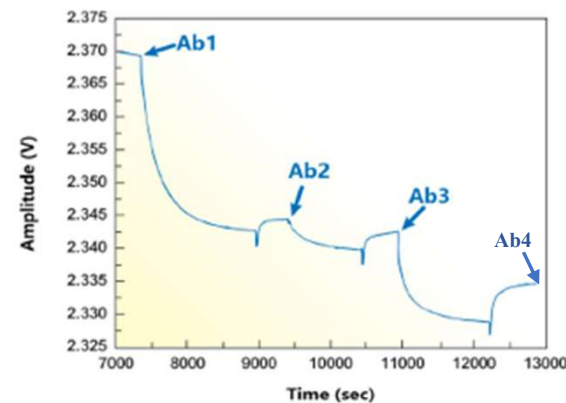


Sandwich Format



Unique Features

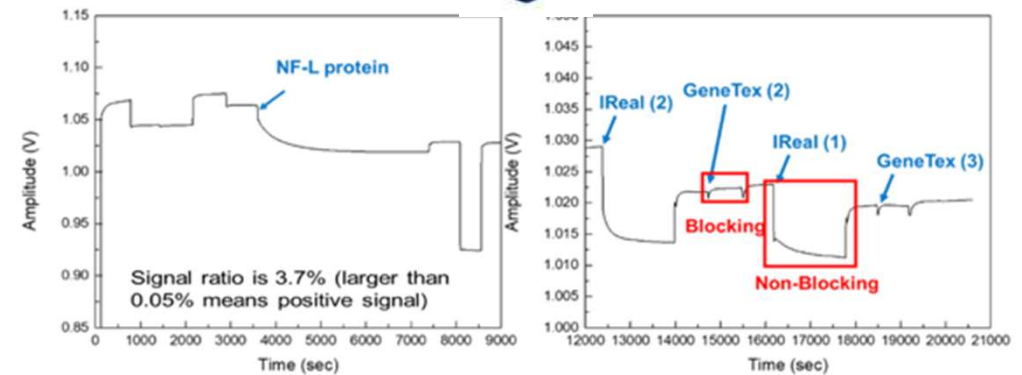
Up to **Four antibody drugs** can be co-localized within the **same 3D space** on a single chip, allowing for the analysis of the same drug target in a simulated intracellular environment."



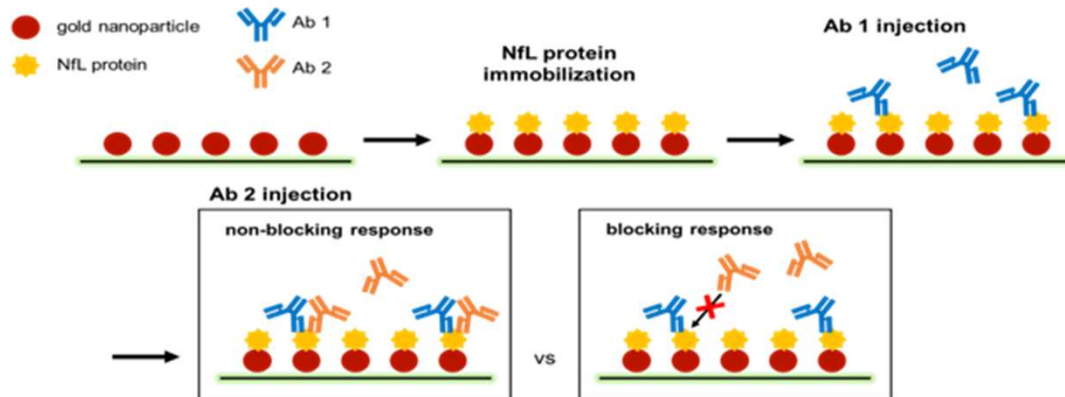
FOPPR Analysis: Epitope Binning

Identifying Equivalents to FDA-Certified Uman Antibody Pair:

Utilizing FOPPR Epitope Binning, we've identified the Abcam (1) and IReal (2) antibodies as potential Uman substitutes. Abcam (1) offers superior stability as a monoclonal antibody, while IReal (2), although polyclonal, showcases remarkable affinity. Consequently, **Abcam (1) is positioned as the capture antibody, and IReal (2) as the detector.**



Tandem Format



NfL Antibody		Second Antibody						
		Abcam (1)	Abcam (2)	GeneTex(1)	GeneTex(2)	GeneTex(3)	IReal (1)	IReal (2)
First Antibody	Abcam (1)							
	Abcam (2)							
	GeneTex(1)							
	GeneTex(2)							
	GeneTex(3)							
	IReal (1)							
	IReal (2)							

blocking
partial blocking
non-blocking

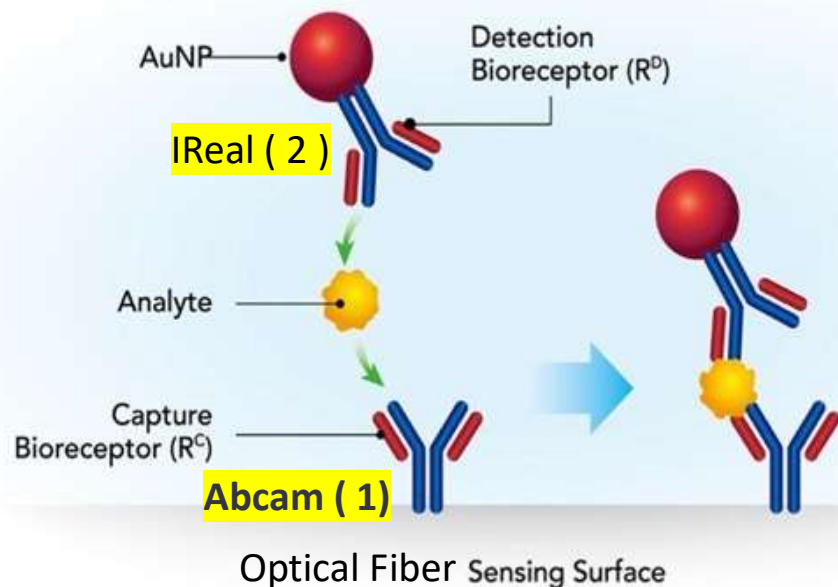
FOPPR Detection: FONLISA NFL Assay

Unique Features

3 Steps: Mix-Incubation 15 mins-Detection 15mins

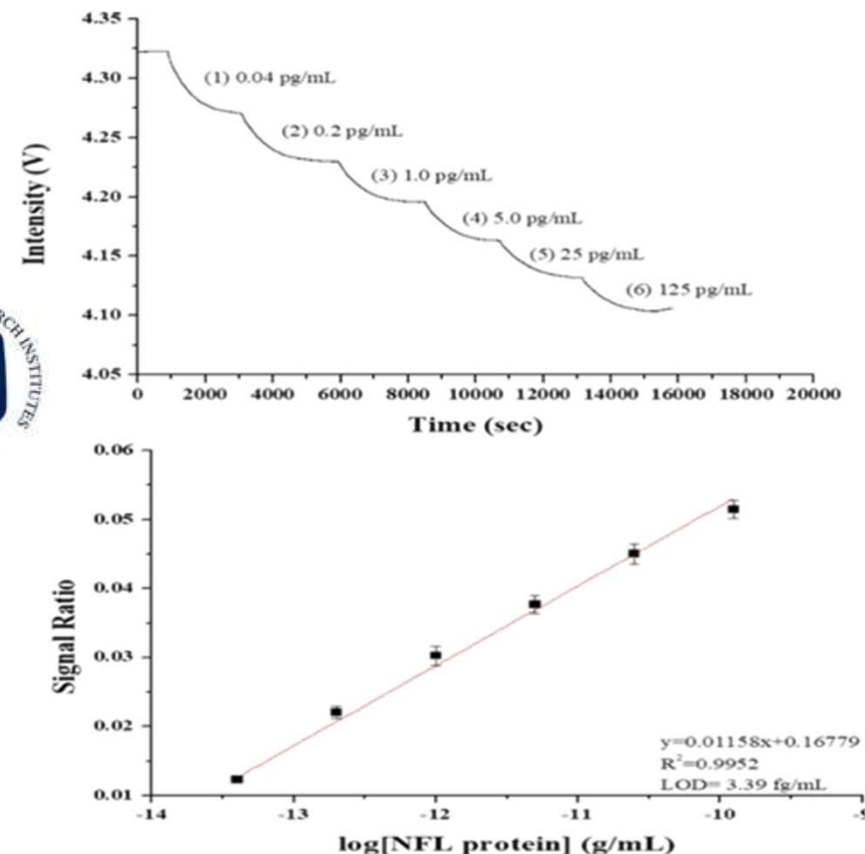
- Ultra-Sensitivity $\sim$ fg/ml
- Wide Linear Range $\sim$ 5 orders
- One Chip for One Clinical Sample
- Cost-effective : No wastage of reagents during each operation.

FONLISA Assay



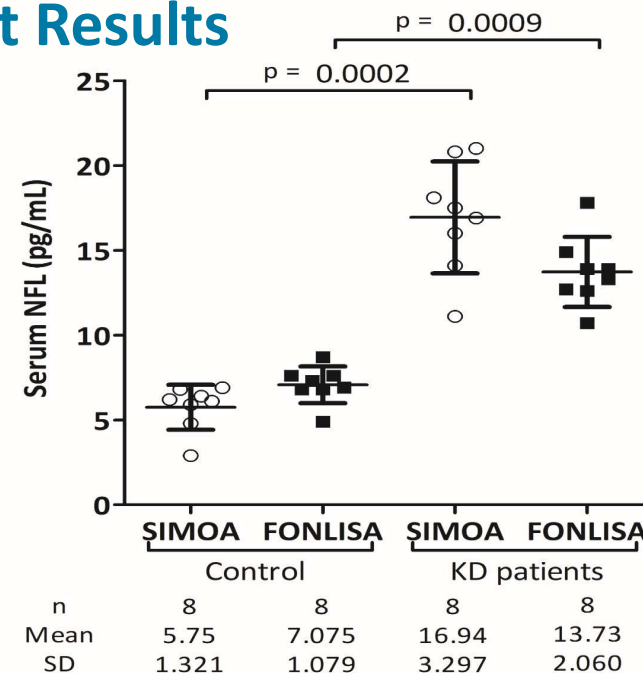
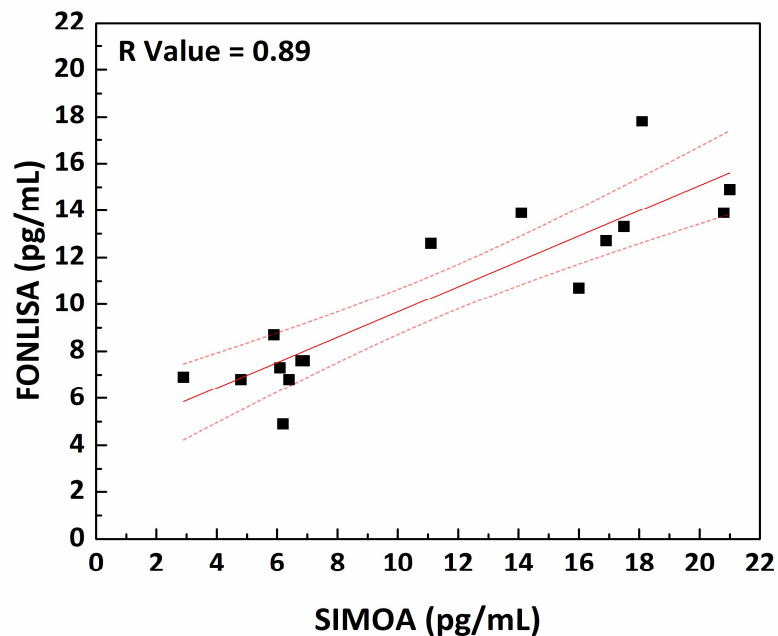
Case Study: Neurofilament light (NFL)

The standard curve demonstrates a linear range of **0.04 pg/mL to 125 pg/mL ($\sim$ 4 orders)**, covering a dynamic range of four orders of magnitude. The limit of detection (LOD) for the Nfl assay was determined to be **24.4 fg/mL**.



FOPPR Detection: FONLISA NFL Assay

Correlation and Significant Results



- The serum NFL concentration correlation variation results between FONLISA and SIMOA methods. (n = 16)
- Red dashed lines are the **95% confidence** intervals for prediction with the regression model.
- **R Value=0.89***

*Explanation of R Value:

0 < |R| < 0.3: Weak correlation

0.3 ≤ |R| < 0.7: Moderate correlation

0.7 ≤ |R| ≤ 1: Strong correlation

This study had developed a novel NFL analysis method FONLISA of FOPPR technique. It demonstrated to be highly sensitive as SIMOA method. The current analyses is primary for the blood serum NFL analysis. It used a small amount of serum sample (volume ≤ 20 µL), and requires low-cost of assay chips and machine. **The NFL analyses results were similar to the most popular use of SIMOA method.**

Clinical samples are still accumulating up to **600+ samples**, and the article has not yet been published.

Centralization



Quanterix

- ✓ Ultra-Sensitivity
- ✓ Wide Linear Range



Quanterix (QTRX.US)
Market Cap: 1B USD
2022 Sales: \$105.5M USD
Instrument price: USD250,000

Decentralization



- ✓ Ultra-Sensitivity $\sim$ fg/ml
- ✓ Wide Linear Range $\sim$ 5 orders
- ✓ Desktop
- ✓ Simple & Easy Handling
- ✓ Cost-effective

The photo was taken at a European medical center's client location.