



**Platform for Quantifying Protein Dynamics
in Real Biological Specimens**

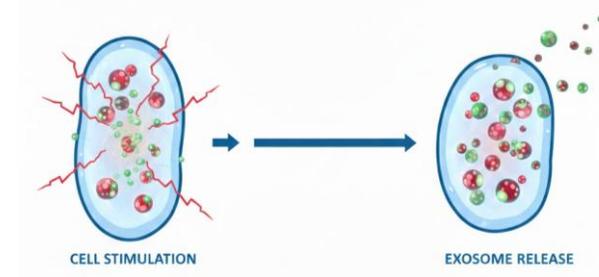
- "Dynamics" "Localization-preserved" "Quantitative"-

The Fundamental Question of Biological Dynamics

Biological phenomena are driven not by *static concentrations*, but by **dynamic molecular changes** triggered by stimuli or treatment.

Cells:

- Produce proteins
- Change their localization
- Load them into exosomes
- Release them to neighboring cells
- Shaping disease progression and treatment response



What researchers truly want to understand is:

“Which proteins are released, when, from where, and in what quantity?”

In other words—the **movement** of biology.

However, until now,

there has been no method capable of quantifying these dynamics directly in real biological specimens.

What Microscopy and ELISA Have Revealed (and What They Cannot Do)

Microscopy (fluorescence, confocal, luminescence)

- Visualizes localization, trafficking, and secretion in real time
- The most intuitive way to observe dynamics

But:

- Low throughput
- Poor quantitation
- Captures individual events, not population-level changes



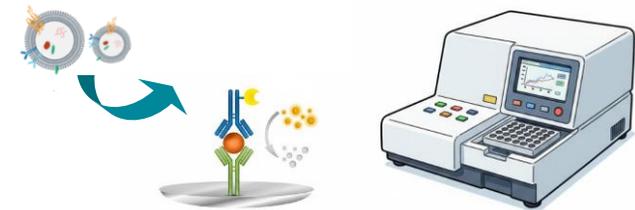
Conventional ELISA

- Can measure samples containing cells or debris
- Allows comparison of pre- and post-stimulation states

But:

- Insufficient sensitivity for trace-level dynamics
- Cannot detect luminal exosome proteins

[Gap] *“Localization-preserved dynamics”*
× “High sensitivity”



Microscopy = “can see but cannot quantify”

ELISA = “can quantify but cannot see trace-level dynamics”

Researchers have long wanted:

“To quantify what microscopy can see—across many samples.”

High-Sensitivity Technologies and the Loss of Biological Dynamics

To detect trace proteins, new technologies emerged:

- **Mass spectrometry**
- **Digital immunoassays**
- **Electrochemiluminescence immunoassays(ECL)**

These dramatically improved sensitivity, but at the cost of **losing biological context**—localization and dynamics.

Mass spectrometry

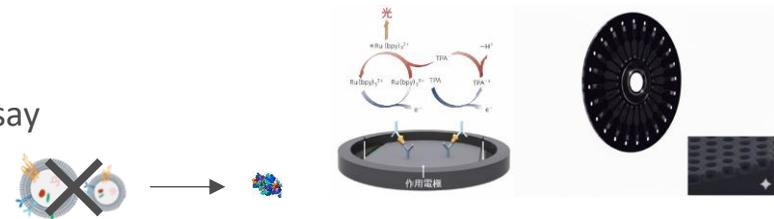
- Requires extraction and homogenization
- Destroys membrane/lumen localization
- Dynamics reduced to “post-extraction concentration differences



Digital immunoassay / Electrochemiluminescence(ECL)

- Require homogeneous liquid samples
- Real specimens containing structures (cells, membranes, exosomes) break the assay
- Localization and structural context are lost

High sensitivity came at the cost of biological meaning.

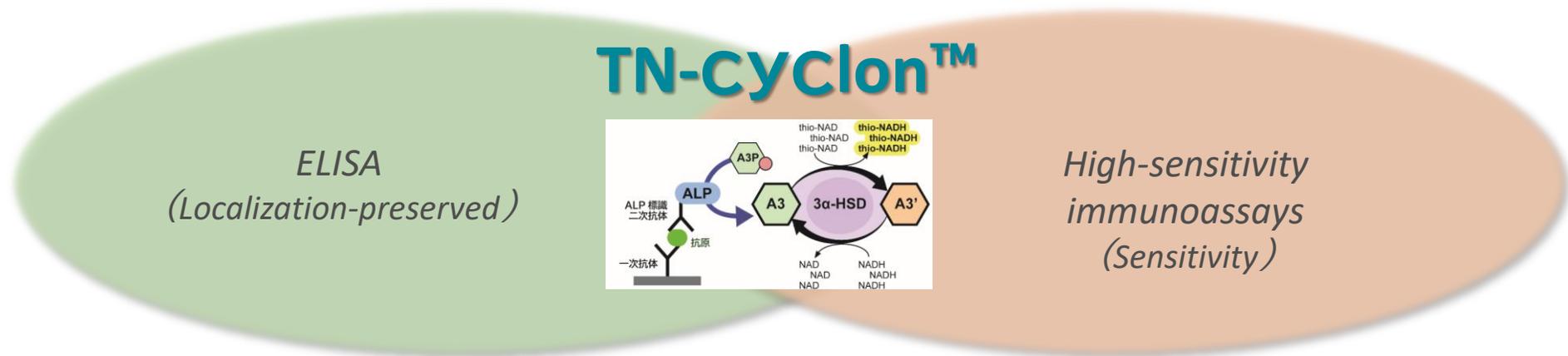


What Was Needed: An “Evolved ELISA”

Researchers needed a new measurement system that:

- Preserves biological structure like ELISA
- Quantifies ultra-trace proteins like high-sensitivity assays
- Captures dynamic changes before/after stimulation
- Retains exosome membrane/lumen localization

Such a technology did not exist—until now.



Mapping the Landscape of Dynamics Technologies

Microscopy → ELISA → High-sensitivity immunoassays

Each has strengths, but none can achieve:

Dynamics × Localization × Ultra-trace quantification × High throughput

TN-cyclon™ uniquely enables all four.

TN-cyclon™ combines:

- ELISA’s ability to preserve localization
- High-sensitivity immunoassays’ ability to quantify trace proteins
- Microscopy-level dynamic insight
- High-throughput scalability

The only platform that quantifies dynamics previously visible only under a microscope—using real specimens.

Technology	Dynamics	Localization	Quantitative	Sensitivity	High Throughput
Microscopy	⊙	⊙	×	×	×
Conventional ELISA	△	○	○	△	⊙
Digital Immunoassay / Electrochemi-luminescence	×	×	⊙	⊙	⊙
TN-cyclon™ (BioPhenoMA)	⊙	⊙	⊙	⊙	⊙

TN-cyclon™: A New Immunoassay for Quantifying Biological Dynamics

TN-cyclon™ is an evolved ELISA designed to:

“Measure ultra-trace proteins while preserving biological localization.”

- Uses BioPhenoMA’s proprietary A3P substrate
- Supported by core patents (JP & international: US/EU/HK)

Key Features

1. Localization preserved

Measures exosome membrane vs. lumen fractions directly

2. Ultra-trace quantification (0.1 pg/mL)

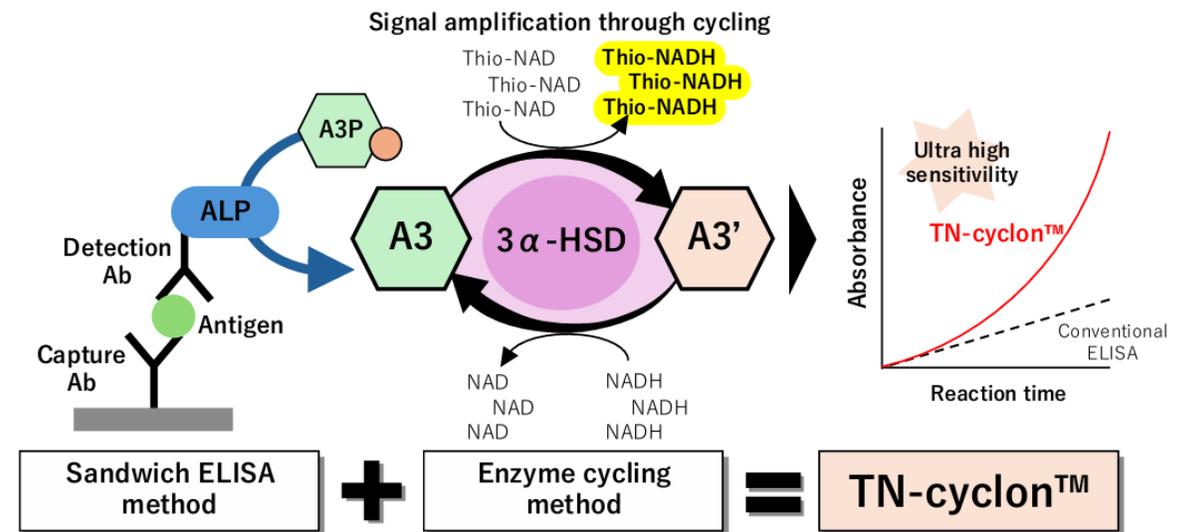
Achieved via enzyme-cycling exponential amplification

3. Standard laboratory equipment

No specialized instruments required

4. High-throughput format

Enables “n-number dynamics analysis” impossible with microscopy



“Localization-preserved × High sensitivity × High throughput”

BioPhenoMA's End-to-End Dynamics Analysis Workflow

BioPhenoMA is the only organization providing the entire workflow required for real-specimen dynamics analysis.

Workflow

- 1. Specimen pre-processing**
(Urine, blood, sputum, exosomes)
- 2. Exosome membrane/lumen fractionation**
Localization preserved
- 3. Ultra-trace quantification (TN-cyclon™)**
- 4. Dynamics analysis**
Pre/post stimulation or treatment
- 5. Mechanistic interpretation & co-research design**



“From sample to mechanism”

Research Example ①: Cancer × Exosome Tumor Marker (GRP78)

Objective

Clarify how exosome membrane/lumen localization relates to disease progression.

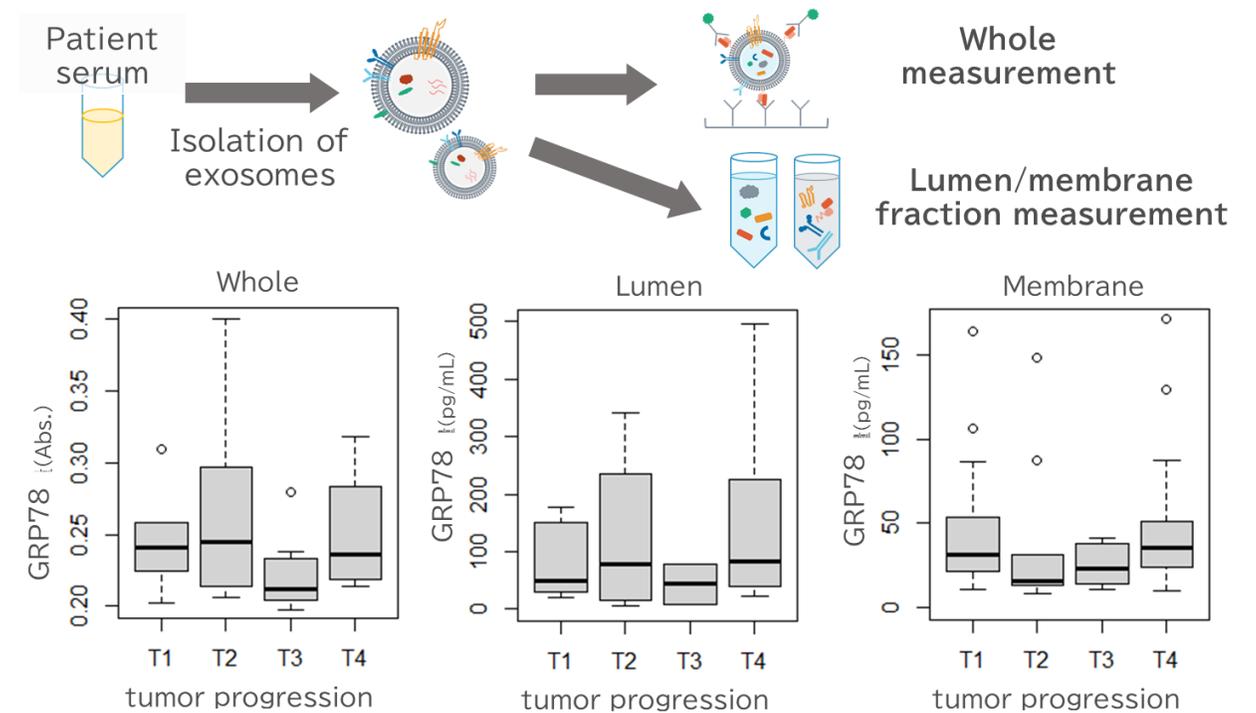
Approach

Exosome extraction → Membrane/lumen fractionation → TN-cyclon™ quantification → Dynamics analysis

Findings

- Luminal GRP78 increases with tumor stage
- After chemotherapy, GRP78-rich exosomes spread horizontally to neighboring cells
- Induce cancer stemness and angiogenesis
-

→ **Revealing mechanisms of treatment resistance**



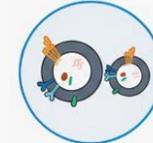
<https://doi.org/10.1016/j.ab.2022.114831>

Six Typical Use Cases for BioPhenoMA

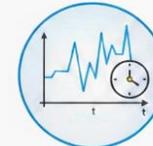
MS/ultrasensitive assays identified candidates, but they fail to reproduce in real specimens



Need to determine whether an Exosome marker acts on the membrane or in the lumen



Clarify the biological basis of responder vs. non-responder differences



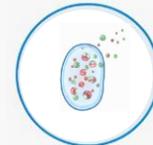
Quantify ultra-trace proteins in real specimens with structural components (urine, sputum, etc.)



Evaluate CDx candidates early using actual biological specimens



Launch new exosome-based research programs in oncology or CNS fields



Positioning by Research Purpose

BioPhenoMA uniquely occupies the space of:

Dynamics × Localization-preserved × Ultra-trace quantification

Purpose	Microscope	ELISA	High-Sensitivity Immunoassay	BioPhenoMA
Localization observation	◎	×	×	○
Dynamics observation	◎	△	×	◎
Ultra-trace quantification	×	△	◎	◎
Localization-preserved measurement	○	○	×	◎
High-throughput processing	×	◎	◎	◎

Summary

BioPhenoMA is the **only platform** enabling real-specimen dynamics analysis.

- Quantifies ultra-trace proteins while preserving biological localization
- Evaluates **localization** × **trace amount** × **dynamics** simultaneously
- Quantifies phenomena previously visible only under a microscope
- Enables early CDx evaluation
- Reveals responder/non-responder mechanisms
- Supports new exosome-based research in oncology and CNS

→ **The only technology that quantifies
“the movement of biology” directly in real specimens.**



Create an innovative technology platform that allows anyone to easily detect ultra-trace proteins anywhere and contribute to further progress in the biomedical field

<https://www.biophenoma.com/en/contact>

Appendix

Existing Technologies: Principles and Limitations (Why They Fail for Dynamics)

Conventional technologies are built on homogenization, extraction, and surface-dependent detection, which inevitably destroy membrane/lumen localization and temporal information.

① Mass Spectrometry (MS)

Principle:

extraction → digestion → ionization → mass detection

- Requires extraction and homogenization
- Destroys exosome membrane/lumen localization
- Dynamics reduced to “post-extraction concentration differences”
→ Detects trace proteins, but loses biological context

(Dynamics × / Localization ×)

② Digital Immunoassay

Principle:

digital photon counting per antibody-coated bead

- Requires homogeneous liquid samples
- Structural components (cells, membranes) disrupt bead behavior
- Preprocessing removes biological structures
→ Trace detection possible, but no localization

(Dynamics × / Localization ×)

③ Electrochemiluminescence Immunoassay (ECL)

Principle:

antibody immobilization and signal detection on electrode surface

- Exosomes/membrane fragments block electron transfer
- Surface-dependent detection breaks down
→ High sensitivity, but cannot handle localization

(Dynamics × / Localization ×)

④ Conventional ELISA

Principle:

solid-phase antibody × washing × colorimetric detection

- Can measure samples containing structures
- But insufficient sensitivity (below pg/mL is difficult)
- Cannot detect luminal exosome proteins
→ Preserves localization, but cannot see trace proteins

(Dynamics △ / Sensitivity ×)

Conventional technologies either “sacrifice localization to measure trace proteins” or “sacrifice trace detection to preserve localization.”

TN-cyclon™ fills this gap.

Flow of the Contract Research Service:

You may request support starting from *any* stage of the workflow.

Case1: Novel Biomarker Discovery

- Provide matched antibody–antigen pairs and sample specimens
- Antibody screening available upon request

[Examples]:

- Already have matched antibodies; need a high-sensitivity assay
- Target protein identified; need to select matched antibodies and build an assay

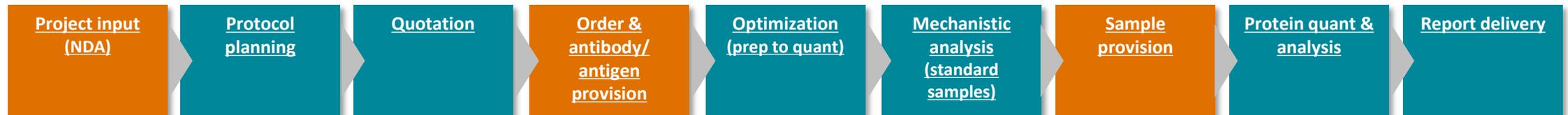
Case 2: Re-evaluation of Existing Biomarkers

- Provide sample specimens (antibodies/antigens also accepted)

[Examples]:

- Multiple known biomarkers identified via proteomics; need behavioral analysis
- Need an EV-based assay system (preprocessing → quantification) and sample measurement

* Flexible Workflow Entry. We can support any step of the workflow depending on your needs. Workflow Steps ■ Provided by Client ■ Performed by BioPhenoMA



If you would like to try TN-cyclon™ yourself, we also offer research-use reagent kits for in-house evaluation.

- PD-L1 TN-cyclon™ ELISA Kit
- TN-cyclon™ ELISA Development Kit

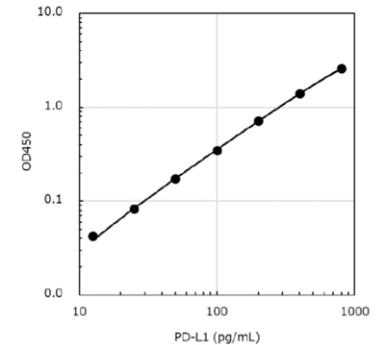
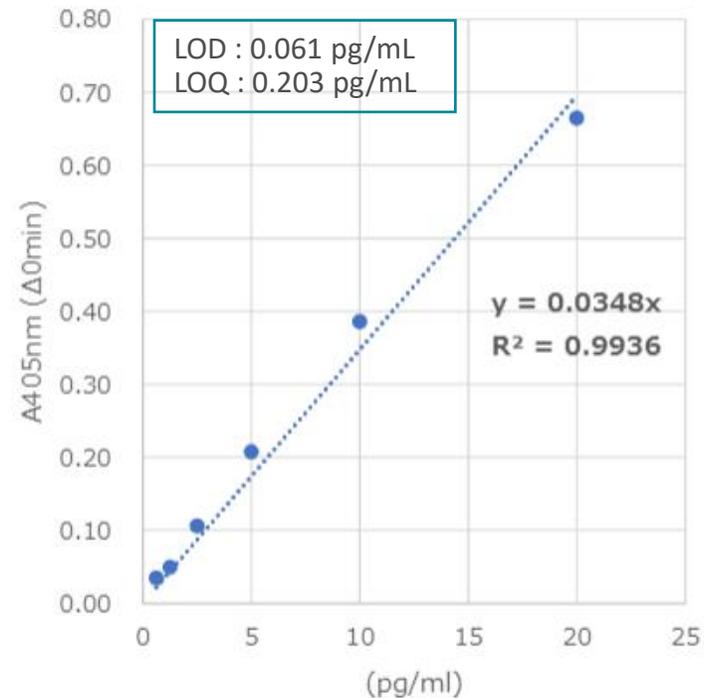
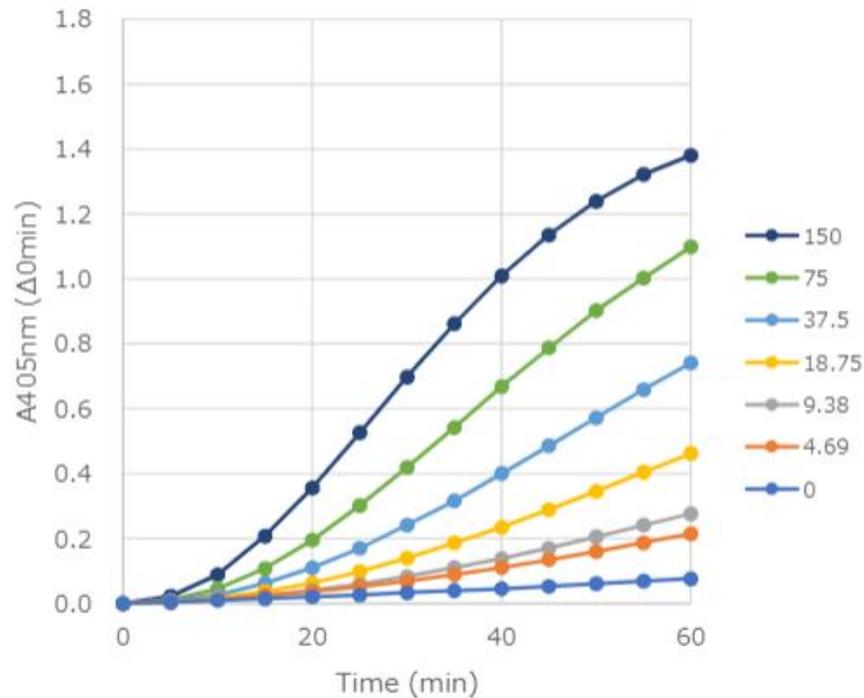


TN-cyclon™ Measurement Example (PD-L1)

Example: PD-L1 (immune checkpoint molecule)

TN-cyclon™ quantifies **0.1 pg/mL-level proteins** using standard laboratory equipment.

- Clear separation between blank and signal (high S/N)
- Achieves **>100-fold higher sensitivity** compared with HRP-ELISA using the same antibody pair



Reference
(HRP-ELISA):
sensitivity: 50 pg/mL
Detection limit: 1 pg/mL

Team



CSO (Chief Scientific Officer)
Co-Founder

Etsuro Ito, Ph.D.

He is one of the inventors of the core technology, the improved enzyme cycling method. After graduating from WASEDA University (majoring in physics and applied physics, with a PhD in science), he served as a visiting fellow at the NIH (National Institutes of Health), an assistant professor at Hokkaido University, and a professor at Tokushima Bunri University, before assuming his current position as a professor at the Faculty of Education and Integrated Arts and Sciences, WASEDA University.



President and CEO

Daisuke Niwa, Ph.D.

He has experience in R&D and commercialization across clinical testing, medical devices, and pharmaceuticals, with strong expertise in the healthcare sector. After completing his doctoral studies at WASEDA University Graduate School, he held assistant and lecturer positions at the WASEDA University Integrated Research Institute for Advanced Science and Healthcare, and later worked at ROHM Co., Ltd. and Teijin Pharma Co., Ltd. He became a Partner at WASEDA University Ventures Co., Ltd. in 2022.



Chief Scientist

Hatsuki Shiga, Ph.D.

She is a scientist with proven experience in national research programs and specialized expertise in advancing enzyme cycling technologies, contributing both technical depth and practical implementation skills. After graduated Ph.D. from Graduate School of Hokkaido University, she worked at JBIC (Japan Bioindustry Informatics Consortium), and as an assistant professor at Fukushima Medical University before becoming Chief Scientist of BioPhenoMA.



Team Leader

Noriko Yamamoto

Pharmacists with a wide range of work experience. After working at a dispensing pharmacy, a pharmaceutical/medical device agency, and overseeing cosmetics/quasi-drugs, he joined BiophenoMA. He is currently furthering his expertise by utilizing the wide range of experience he has gained up to that point.



Technical Adviser

Teruki Yoshimura, Ph.D.

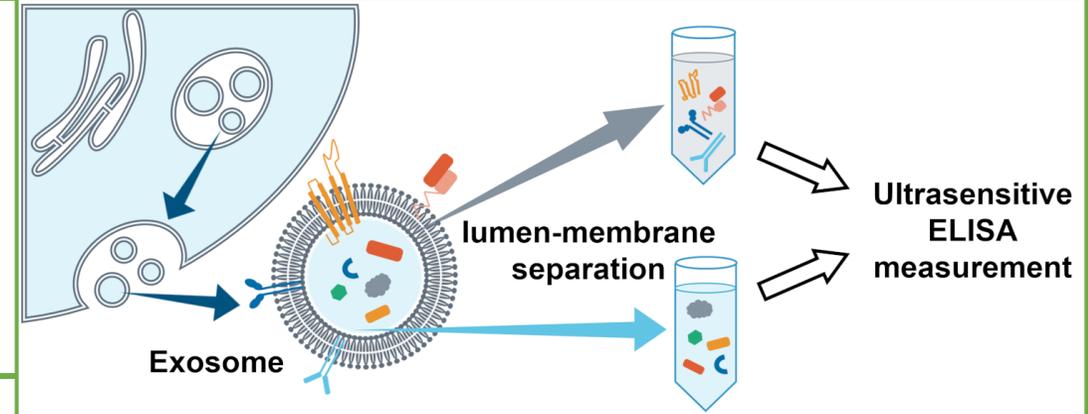
He is one of the inventors of the core technology, the improved enzyme cycling method. After graduating from Hokkaido University Graduate School (PhD in Pharmacy), he studied at Higashinohon Gakuen University and New York University. Visiting Researcher at the Medical Center Former Associate Professor and now a Professor at the Health Sciences University of Hokkaido.

Appendix

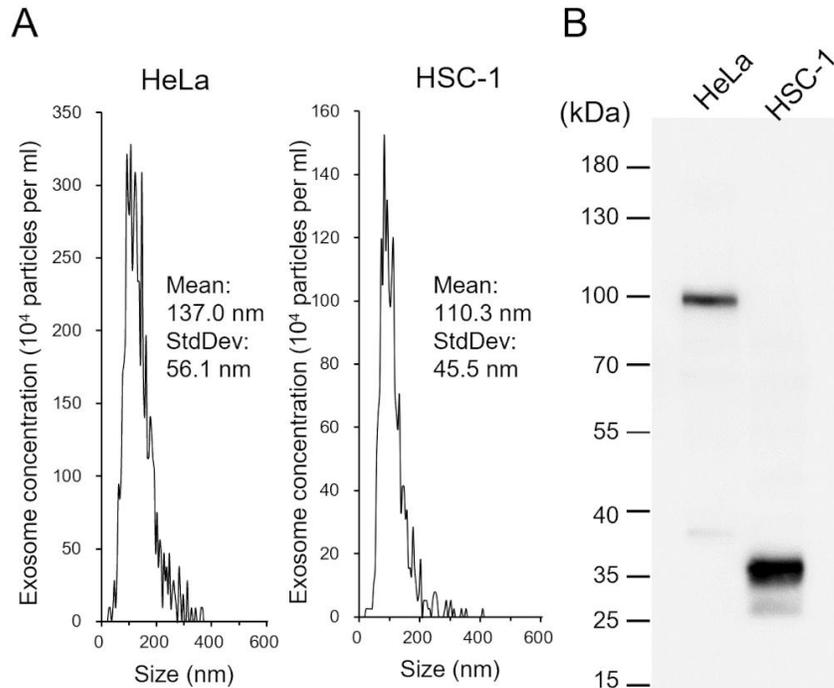
Exosomes × Cancer

◆ Measuring proteins by membrane and lumen fractionation of exosomes

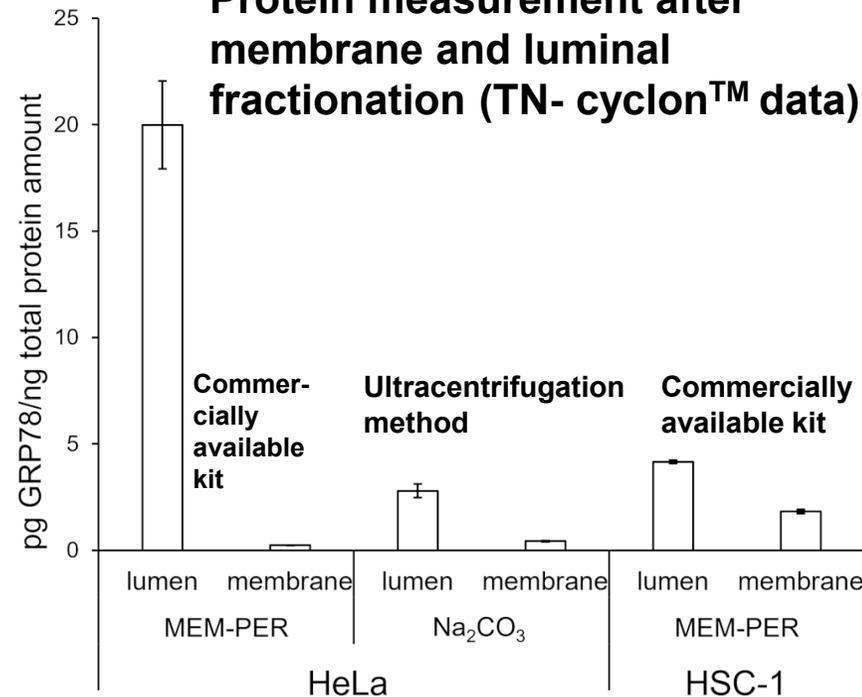
- (1) **Exosome collection:** Ultracentrifugation is a rare method worldwide, and the yield is very low. We collect exosomes using a commercially available kit. The size of the exosomes is confirmed separately. Even if the purity of the exosomes is low, the specificity of the target protein is ensured by using antibodies in TN-cyclon™.
- (2) **Membrane/lumen fractionation:** Using a cell membrane/lumen fractionation kit, membrane proteins and luminal proteins are separated and measured.



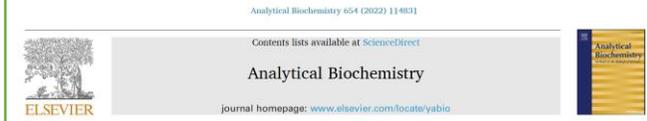
Exosome collection



Protein measurement after membrane and luminal fractionation (TN- cyclon™ data)



The role of exosomes is currently attracting attention in elucidating the mechanisms of neurodegenerative diseases (Parkinson's disease and Alzheimer's disease), and we are accepting contract research from domestic and international companies using patient samples to measure exosomes after fractionating them.



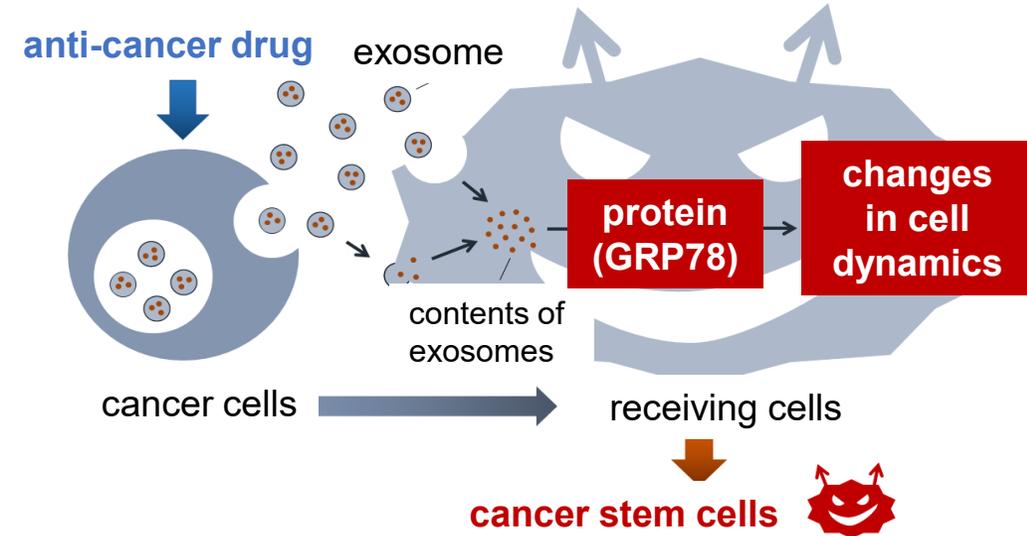
Ultrasensitive ELISA detection of proteins in separated lumen and membrane fractions of cancer cell exosomes

Kanako Iha^a, Naoko Tsurusawa^a, Hsin-Yi Tsoi^{b,c}, Ming-Wei Lin^{b,d}, Hikaru Sonoda^e, Satoshi Watabe^f, Teruki Yoshimura^g, Etsuro Ito^{h,i,j,k}

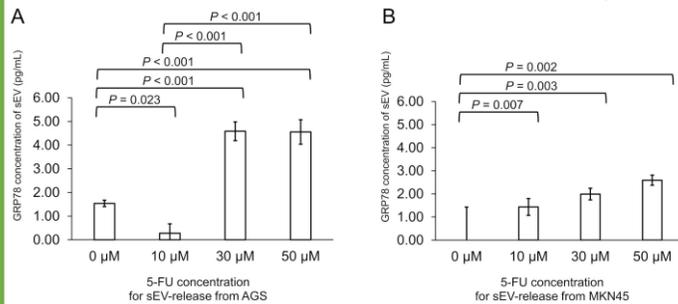
^aDepartment of Biology, Waseda University, Shinjuku, Tokyo, 162-8480, Japan
^bDepartment of Medical Research, E-Do Hospital, E-Do Cancer Hospital, Kaohsiung, 82445, Taiwan
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^dDepartment of Nursing, College of Medicine, Foshan University, Kaohsiung, 82445, Taiwan
^eHakari Inc., Bunkyo, Chiba, 562-0065, Japan
^fWaseda Research Institute for Science and Engineering, Waseda University, Shinjuku, Tokyo, 169-8555, Japan
^gSchool of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Tokeino, Ishikari, Hokkaido, 061-0293, Japan
^hGraduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, 80706, Taiwan

◆ Are anticancer drugs cancer-promoting drugs?

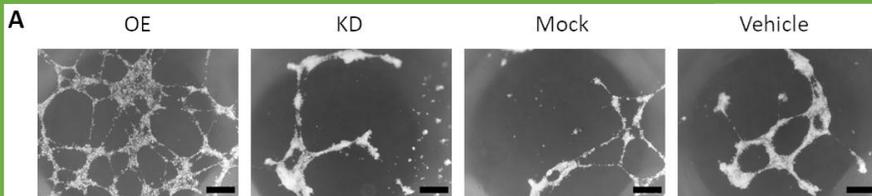
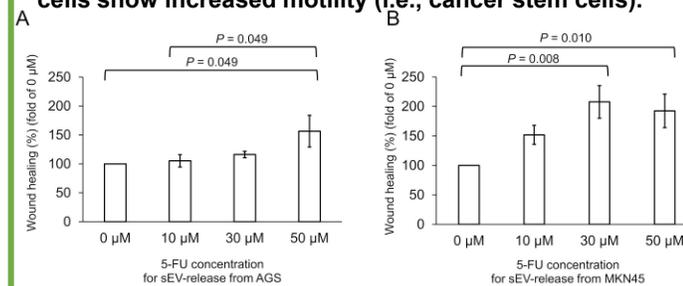
- Horizontal transmission of information via exosomes:** When gastric cancer cells are treated with an anticancer drug (5-FU), they secrete exosomes containing a protein called GRP78.
- Cancer stem cell transformation:** Cancer cells that receive exosomes rich in GRP78 transform into cancer stem cells, which means that they become resistant to anticancer drugs and the cancer worsens.
- Angiogenesis:** Vascular endothelial cells that receive exosomes rich in GRP78 begin to form blood vessels, i.e., the exosomes begin to attract blood vessels to the tumor microenvironment.
- Investigation of inhibitors:** As it has become clear that GRP78 is associated with the progression of cancer, we are currently searching for inhibitors for GRP78.



As the dose of anticancer drugs increases, the amount of GRP78 contained in exosomes also increases (TN-cyclon™).



When exosomes obtained from high-dose anticancer drug-administered cells were applied to the other cells, these cells show increased motility (i.e., cancer stem cells).



When exosomes rich in GRP78 are administered to vascular endothelial cells (the leftmost one contains a lot of GRP78), angiogenesis is observed.



Ultrasensitive Detection of GRP78 in Exosomes and Observation of Migration and Proliferation of Cancer Cells by Application of GRP78-Containing Exosomes

Naoko Tsurusawa¹, Kanako Iha¹, Akane Sato¹, Hsin-Yi Tsai^{2,3}, Hikaru Sonoda⁴, Satoshi Watabe⁵, Teruki Yoshimura⁶, Deng-Chyang Wu^{7,8}, Ming-Wei Lin^{3,5,9,*} and Etsuro Ito^{1,10,*}



Gastric Cancer Cell-Derived Exosomal GRP78 Enhances Angiogenesis upon Stimulation of Vascular Endothelial Cells

Kanako Iha¹, Akane Sato¹, Hsin-Yi Tsai^{2,3}, Hikaru Sonoda⁴, Satoshi Watabe⁵, Teruki Yoshimura⁶, Ming-Wei Lin^{2,7,8,*} and Etsuro Ito^{1,3,9,*}

Citation: Tsurusawa, N.; Iha, K.; Sato, A.; Tsai, H.-Y.; Sonoda, H.; Watabe, S.; Yoshimura, T.; Wu, D.-C.; Lin, M.-W.; Ito, E. Ultrasensitive Detection of GRP78 in Exosomes and Observation of Migration and Proliferation of Cancer Cells by Application of GRP78-Containing Exosomes. *Cancers* 2022, 14, 3887. <https://doi.org/10.3390/cancers14163887>

Citation: Iha, K.; Sato, A.; Tsai, H.-Y.; Sonoda, H.; Watabe, S.; Yoshimura, T.; Lin, M.-W.; Ito, E. Gastric Cancer Cell-Derived Exosomal GRP78 Enhances Angiogenesis upon Stimulation of Vascular Endothelial Cells. *Curr. Issues Mol. Biol.* 2022, 44, 6145–6157. <https://doi.org/10.3390/cimb44120419>

Appendix

Infection diseases

Research Example ②: Infectious Disease (Tuberculosis)

Preprocessing method development × protein marker detection

Objective:

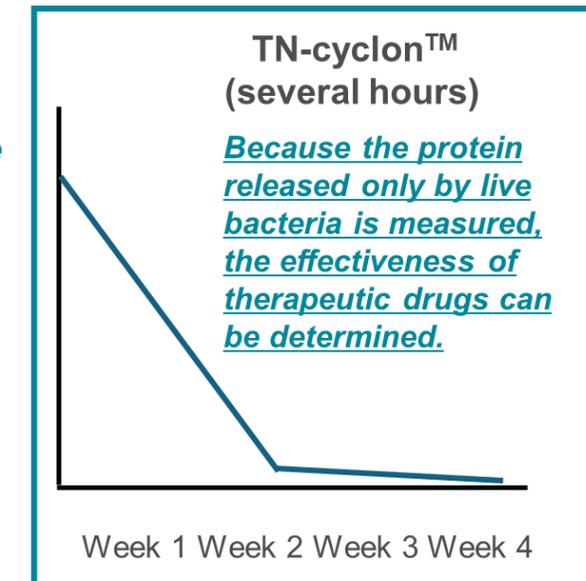
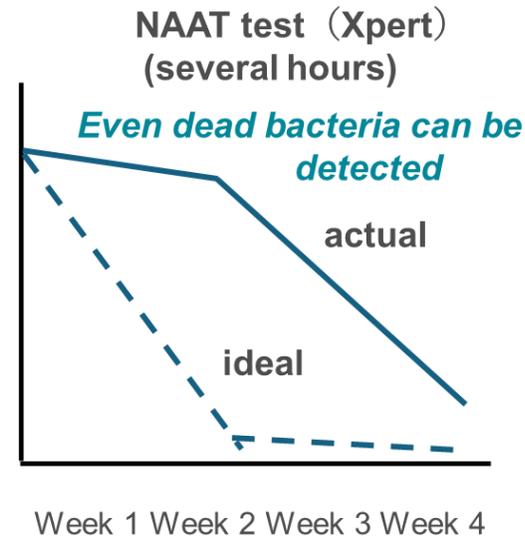
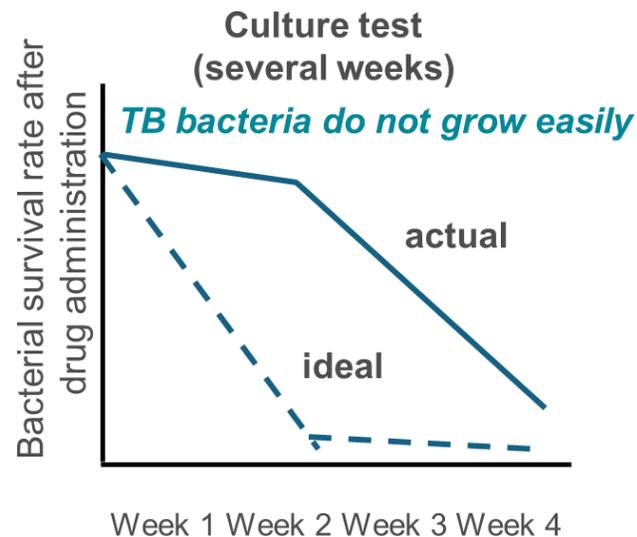
Rapidly determine whether live *Mycobacterium tuberculosis* remains after treatment.

Approach:

Develop preprocessing → detect trace proteins

Findings:

Live-bacteria-derived proteins enable treatment efficacy assessment within hours.



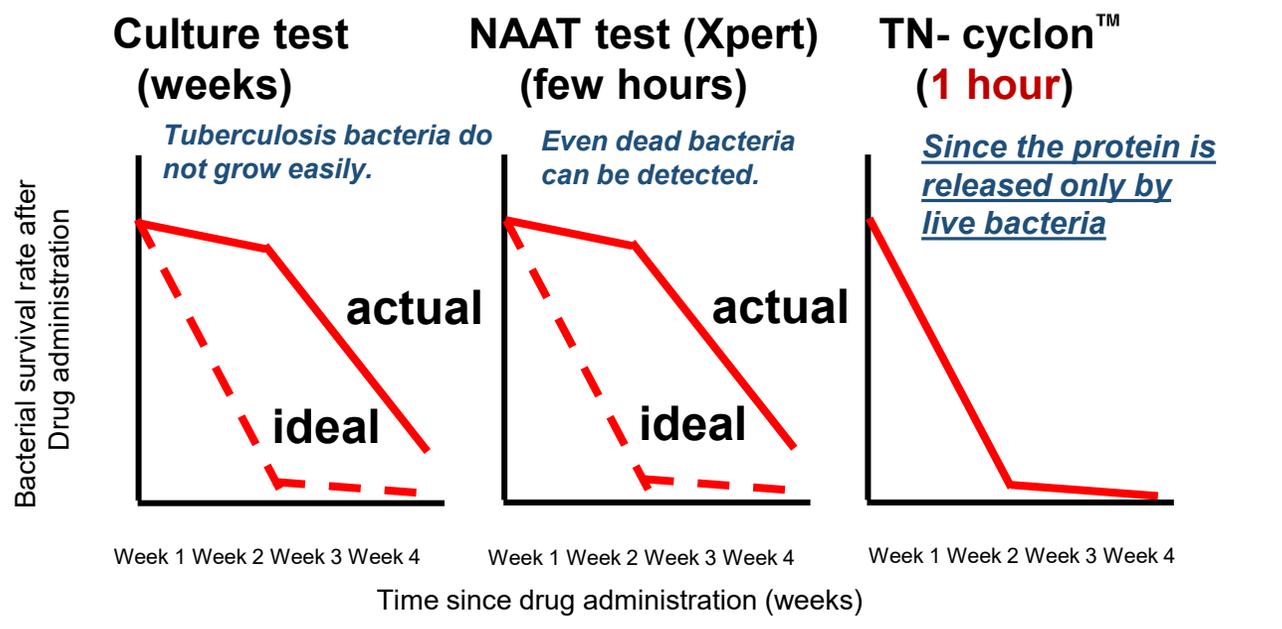
Time since drug administration (weeks)

◆ Tuberculosis: Tuberculosis (TB) testing using TN-cyclon™

- (1) **Culture test:** Although highly reliable, it takes a long time, from **10 days to 6 weeks, to make a diagnosis**. It is still the **definitive diagnosis**.
- (2) **Smear test:** This is useful as a same-day test. However, because **its sensitivity and specificity are insufficient**, it should be used as a screening test.
- (3) **NAAT test:** This is useful as a same-day test. However, it requires expensive equipment, a complex protocol, and an air-conditioned room. Furthermore, it is less sensitive than culture testing, so there is a risk that positive patients will not be found with this PCR testing. Furthermore, **it may detect dead bacteria**.
- (4) **TN-cyclon™:** This measures the protein MPT-64 as the target. Measurements can be performed in one hour with the same measurement sensitivity as culture tests. Because it measures protein, **only live bacteria are detected**.

Results of one-hour measurement of MPT-64 using TN-cyclon™

blank	0.0703		
SD	0.0005		
3 SD	0.0014	10SD	0.005
LOD		LOQ	
(pg/mL)	0.058	(pg/mL)	0.194
LOD	2.33×10^{-19}	LOQ	7.78×10^{-19}
(moles/assay)		(moles/assay)	



A water-soluble filter is attached to the mask, which adsorbs tuberculosis bacteria contained in exhaled aerosols.

The water-soluble filter is ultrasonically disrupted and the tuberculosis bacteria are collected by centrifugation.

How long patients will need to wear masks remains to be determined.

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Contents lists available at ScienceDirect

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Research paper

A novel, rapid (within hours) culture-free diagnostic method for detecting live *Mycobacterium tuberculosis* with high sensitivity

Wen-Hung Wang^{1,2}, Rikiya Takeuchi^{3,4}, Shu-Huei Jain⁵, Yong-Huang Jiang⁶, Sonoko Watanuki⁷, Yoshiharu Ohtaki⁸, Kazunari Nakaishi⁹, Satoshi Watabe¹⁰, Po-Liang Lu^{11,12}, Etsuro Ito^{13,14}

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Commentary

Culture-free proof of *Mycobacterium tuberculosis* - a new assay for viable bacteria

Jan Heyckendorf^{1,2}, Stephen H. Gillespie³, Morten Ruhwald⁴

¹Division of Clinical Infectious Diseases, Research Center Borstel, Borstel, Germany

²German Center for Infection Research (GIR), Germany

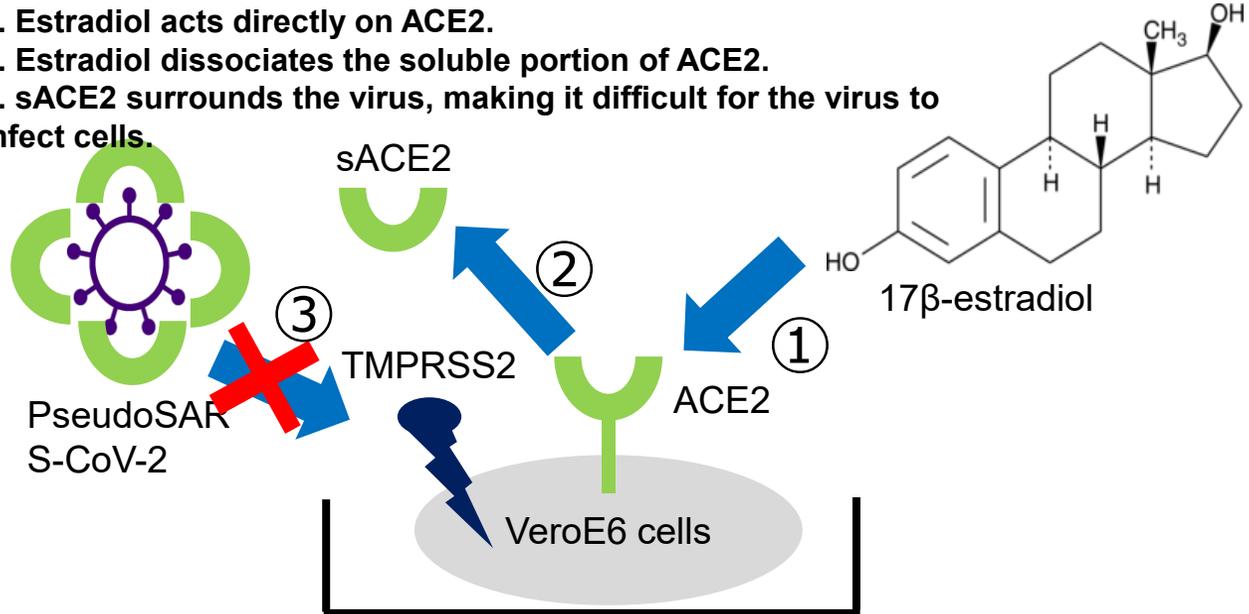
³School of Medicine, University of St. Andrews, St Andrews UK

⁴Foundations of Innovative New Diagnostics, Geneva, CH Switzerland

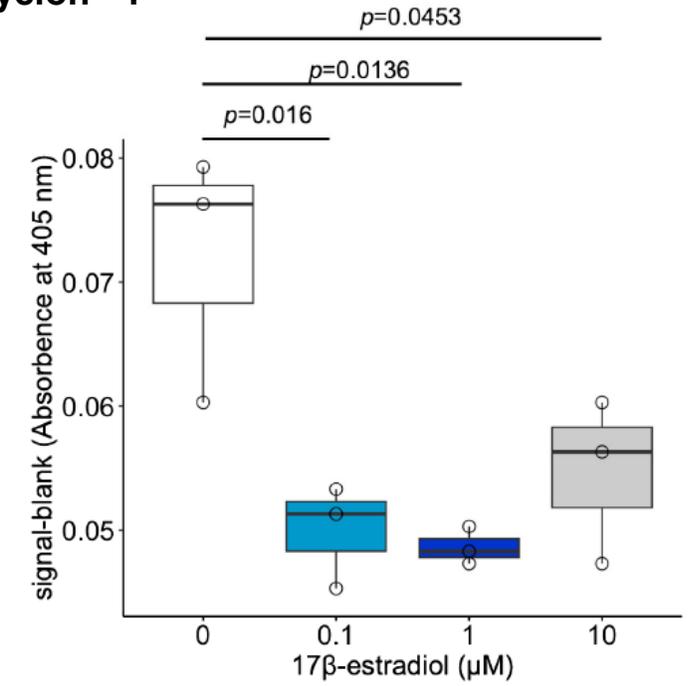
◆ Why do women have a lower infection rate from COVID-19?

- (1) **Women of all races have a lower infection rate:** According to the results of an American survey, women of all races, whether Asian, Black, or White, are less likely to contract COVID-19 than men.
- (2) **Influence of female hormones:** Although various factors such as diet and smoking are considered, we focused on the effects of a female hormone (estradiol).
- (3) **Estradiol dissociates the soluble portion of ACE2:** The virus, SARS-CoV-2, causes infection when its spike protein binds to ACE2 (angiotensin-converting enzyme 2). Through extensive research using TN-cyclon™, it was discovered that estradiol dissociates the soluble portion of ACE2 (sACE2), and the dissociated sACE2 then binds to the viral spike proteins, making it less susceptible to infection.

- 1. Estradiol acts directly on ACE2.
- 2. Estradiol dissociates the soluble portion of ACE2.
- 3. sACE2 surrounds the virus, making it difficult for the virus to infect cells.



Estradiol causes sACE2 to dissociate and surround the virus, resulting in a decrease in the amount of active spike proteins (as measured by TN-cyclon™).



1842

Biol. Pharm. Bull. 46, 1842-1845 (2023)

Vol. 46, No. 12

Note

Removal of Soluble ACE2 in VeroE6 Cells by 17β-Estradiol Reduces SARS-CoV-2 Infectivity

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Appendix

Non-invasive Measurement

Research Example ③: Metabolism (Urinary Adiponectin)

Urine specimens × Establishing thresholds in healthy individuals

A study demonstrating that **urinary adiponectin levels strongly correlate with CKD progression** caused by diabetic nephropathy, suggesting its potential as a new diagnostic marker.

Objective

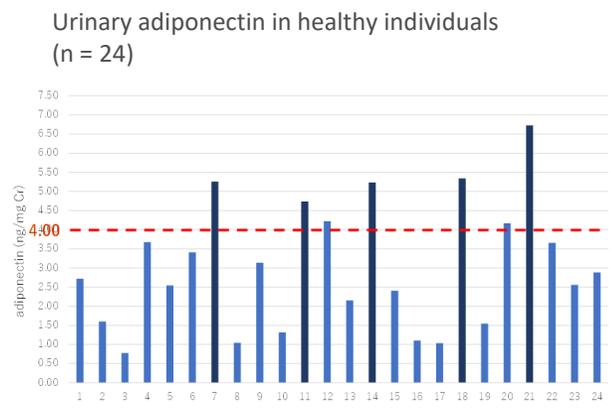
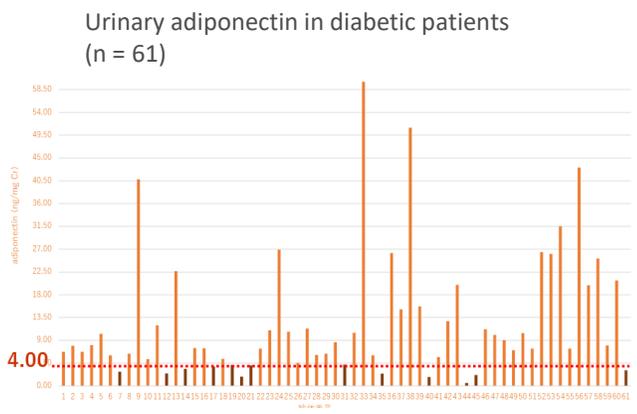
Evaluate the clinical significance of ultra-trace urinary proteins.

Approach

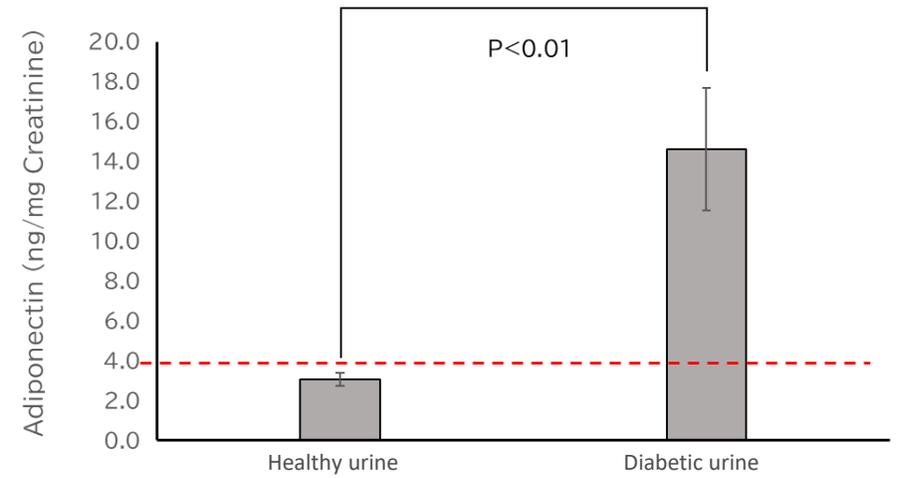
High-sensitivity quantification → Threshold determination

Findings

Urinary adiponectin shows a strong correlation with CKD progression.

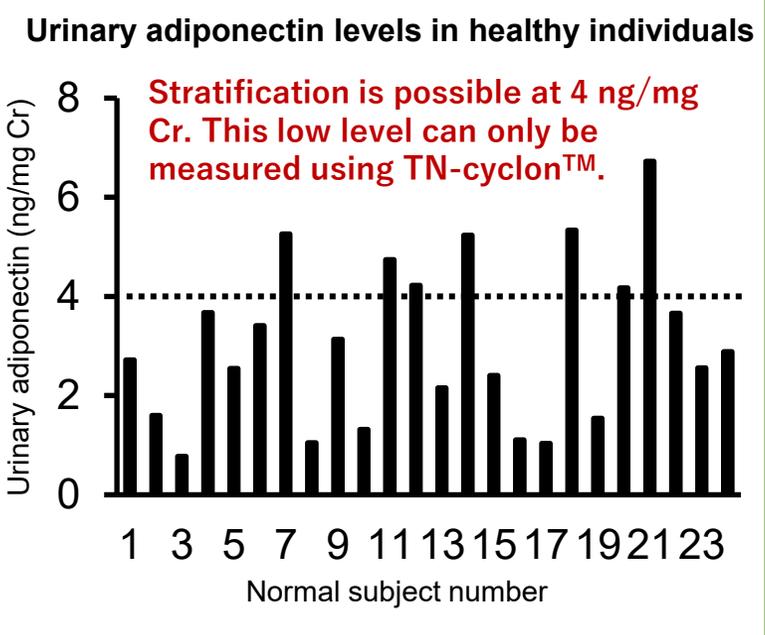
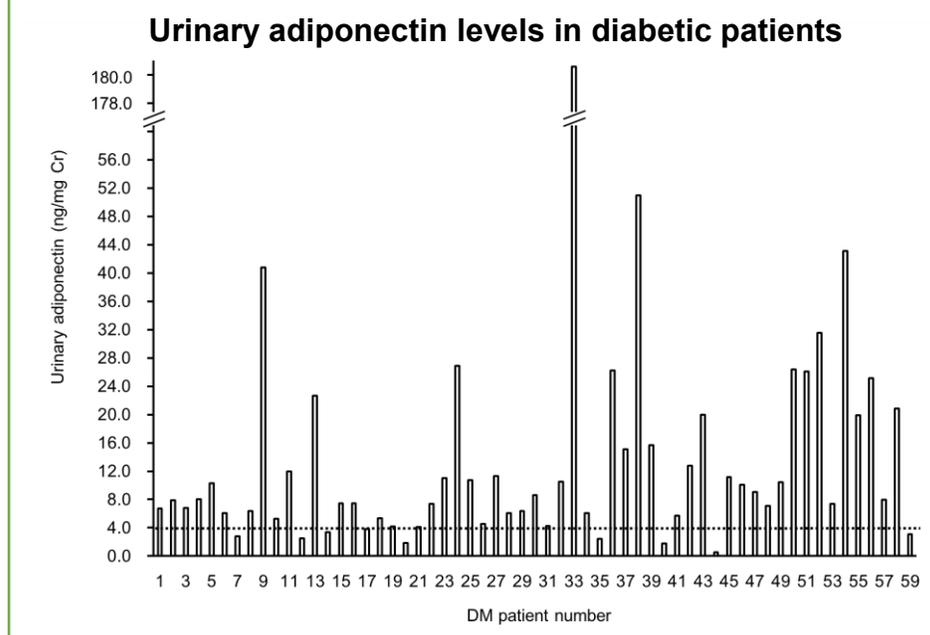
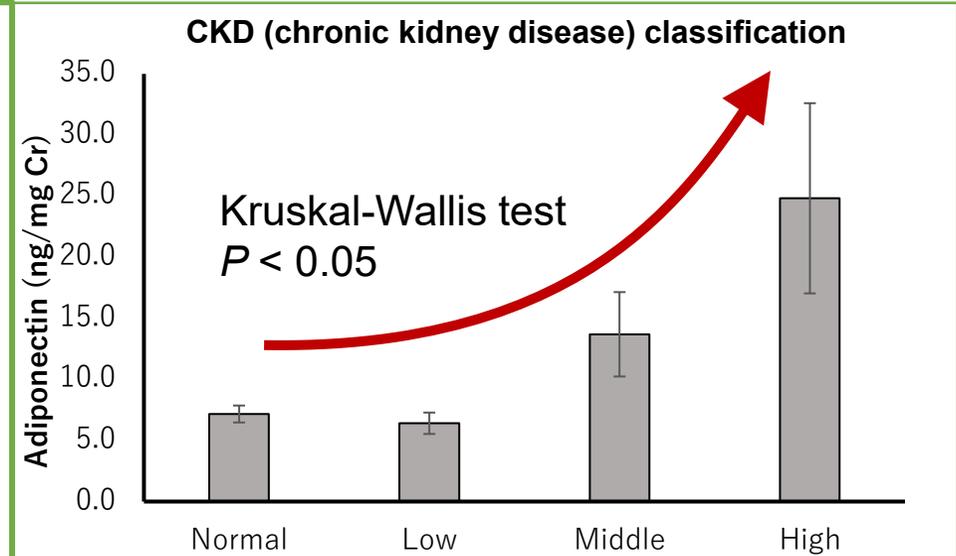


Adiponectin Levels in Urine: Healthy vs. Diabetic Patients



◆ Adiponectin: Measuring what could not be measured before

- (1) **What do ultra-high-sensitivity measurements enable?** Once the disease has progressed, it is too late to confirm that specific protein levels are elevated. Ultra-high sensitive measurement (TN- cyclon™) makes it possible to measure low levels in healthy individuals.
- (2) **Change in urinary adiponectin:** Adiponectin in the blood is known as a 'good hormone', with levels high in healthy individuals and decreasing in those with obesity or diabetes. In other words, it is a good biomarker for lifestyle-related diseases. However, according to research by Ito et al., urinary adiponectin exhibits the opposite change. That is, it is extremely low in healthy individuals and increases as diabetic kidney damage progresses. Therefore, measuring urinary adiponectin levels can indicate the worsening of lifestyle-related diseases.



Prognosis of CKD by GFR and Albuminuria Categories: KDIGO 2012

GFR categories (ml/min/1.73 m ²) Description and range	GFR	Normal or high	Persistent albuminuria categories Description and range		
			A1	A2	A3
			Normal to mildly increased	Moderately increased	Severely increased
G1	≥90	Normal	Low	High	
G2	60-89	Low	Middle	High	
G3a	45-59	Middle	High	High	
G3b	30-44	High	High	High	
G4	15-29	High	High	High	
G5	<15	High	High	High	

Open access Research

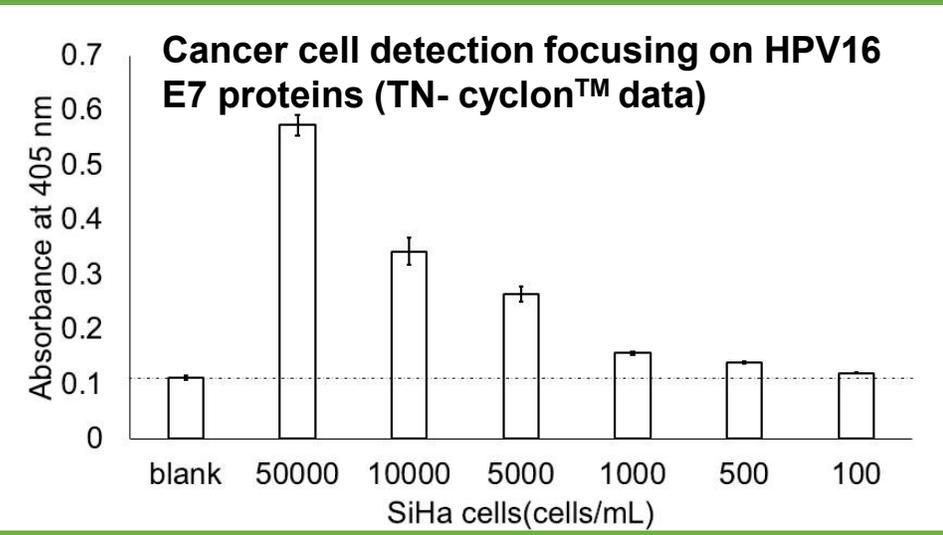
BMJ Open Diabetes Research & Care Urinary adiponectin as a new diagnostic index for chronic kidney disease due to diabetic nephropathy

To cite: Yamakado S, Cho H, Inada M, et al. Urinary adiponectin as a new diagnostic index for chronic kidney disease due to diabetic nephropathy. *BMJ Open Diab Res Care* 2019;7:e000661. doi:10.1136/bmjdc-2019-000661

Shinnosuke Yamakado,¹ Hiroki Cho,¹ Mikio Inada,¹ Mika Morikawa,² Yong-Huang Jiang,² Kenji Saito,² Kazunari Nakaiishi,² Satoshi Watabe,² Hitomi Takagi,² Mughso Kanada,² Akira Nakatsuma,² Masaki Niromiya,³ Hitomi Imachi,⁴ Takeshi Arai,⁴ Takuo Yoshimoto,⁴ Koji Murao,⁴ Jun-Hao Chang,⁵ Shih-Min Chen,⁶ Yi-Chen Shih,⁶ Min-Jing Zeng,⁶ Liang-Yin Ke,⁶ Chiu-Huang Chen,⁶ Teruki Yoshimura,⁷ Toshiaki Miura,⁷ Etsuro Ito^{1,8}

◆ Cervical cancer screening using urine

- (1) **Cervical cancer:** Cervical cancer is both a cancer and an **infectious disease**, and global health calls are being made to eradicate it.
- (2) **High hurdles:** Cervical cancer can be prevented through vaccines and screening, but for unmarried women, gynecological screening is a high hurdle, and we must somehow find ways to reduce this.
- (3) **Urine testing:** A small amount of vaginal discharge is always mixed with urine. Therefore, it is expected that cervical cancer cells will also be mixed in urine, and we focused on the possibility that cervical cancer screening may be possible by testing urine.
- (4) **High-risk types of HPV:** The main cause of cervical cancer is HPV infection, and we focused on the high-risk type HPV 16 in particular.



Noninvasive Detection of HPV16 Oncogenic Activity in Urine Using Ultrasensitive ELISA

Oncogenic activity of human papillomavirus (HPV) plays a critical role in the development and progression of cervical cancer

Selection of urine samples by uniplex E6/E7 polymerase chain reaction

Classification of samples by cervical intraepithelial neoplasia (CIN) grade using cervical biopsy results

Ultrasensitive ELISA
- Developed to detect HPV16 E7 oncoprotein levels
- Detects cells at >500 cells/mL but not HPV18-positive cells

Current screening methods, such as Pap test, are invasive and detect HPV DNA, but detection of HPV proteins in urine is noninvasive and may offer better insights

CIN Grade	Percentage Positive	Urine Samples Positive
CIN1: Low-grade lesions	80%	4/5 of urine samples were positive for E7 oncoproteins
CIN2: Moderate-grade lesions	71%	5/7 of urine samples were positive for E7 oncoproteins
CIN3: High-grade lesions	38%	3/8 of urine samples were positive for E7 oncoproteins

Urine samples with undetectable E7 oncoproteins

Negative for live HPV16-positive cells/inactive state of infection

ELISA: Enzyme-linked immunosorbent assay; DNA: Deoxyribonucleic acid

Ultrasensitive ELISA can effectively quantify HPV16 E7 oncoproteins in urine, aiding noninvasive cervical cancer screening

- HPV16 was positive in 80% of urine samples from patients with CIN1, an early stage of precancerous disease, 71% of urine samples from patients with CIN2, and 38% of urine samples from patients with CIN3.
- There is less resistance to collecting and submitting urine samples yourself than to going to a hospital for a checkup and seeing a doctor, and if the barriers to screening are lowered, it could be a step towards eradicating cervical cancer.

Article
Quantification of HPV16 E7 Oncoproteins in Urine Specimens from Women with Cervical Intraepithelial Neoplasia

Daiki Makioka ^{1,†}, Mikio Inada ^{1,†}, Masayuki Awano ¹, Ema Saito ¹, Takuya Shinoda ¹, Satoko Abe ¹, Teruki Yoshimura ², Martin Müller ^{3,*}, Toshiyuki Sasagawa ^{3,*} and Etsuro Ito ^{1,3,*}

Citation: Makioka, D.; Inada, M.; Awano, M.; Saito, E.; Shinoda, T.; Abe, S.; Yoshimura, T.; Müller, M.; Sasagawa, T.; Ito, E. Quantification of HPV16 E7 Oncoproteins in Urine Specimens from Women with Cervical Intraepithelial Neoplasia. *Microorganisms* **2024**, *12*, 1205. <https://doi.org/10.3390/microorganisms12061205>

Appendix

Publication List

Research Achievements (Publications)

We have an extensive track record in mechanistic analysis using real biological specimens.

[\[Malaria\]](#)

Ultrasensitive ELISA for Accurate Detection of Plasmodium falciparum Infection

Open Forum Infectious Diseases, Volume 12, Issue 12, 2025, ofaf711, <https://doi.org/10.1093/ofid/ofaf711>

[\[Cancer & Infectious Disease \(GRP78\)\]](#)

GRP78: A Multifaceted Role in Cancer Progression and Infectious Disease Transmission

Journal of Cellular Immunology 2025, 7(1), 9-13, <https://doi.org/10.33696/immunology.7.217>

[\[Respiratory Infections \(Influenza, etc.\)\]](#)

Ultrasensitive protein-level detection for respiratory infectious viruses.

Frontiers in Immunology 2024, 15(12), 1445771., <https://doi.org/10.3389/fimmu.2024.1445771>

[\[Cervical Cancer\]](#)

Quantification of HPV16 E7 Oncoproteins in Urine Specimens from Women with Cervical Intraepithelial Neoplasia,

Microorganisms 2024, 12(6), 1205, <https://www.mdpi.com/2076-2607/12/6/1205>

[\[Dengue Fever\]](#)

Advanced detection method for dengue NS1 protein using ultrasensitive ELISA with thio-NAD cycling.

Viruses, 2023, 15(9), 1894., <https://doi.org/10.3390/v15091894>

[\[Gastric Cancer \(Exosomes\)\]](#)

Gastric cancer cell-derived exosomal GRP78 enhances angiogenesis upon stimulation of vascular endothelial cells.

Current Issues in Molecular Biology, 2022, 44(12), 6145–6157., <https://doi.org/10.3390/cimb44120419>

[\[SARS-CoV-2 Spike Protein\]](#)

Ultrasensitive detection of SARS-CoV-2 spike proteins using the thio-NAD cycling reaction: A preliminary study before clinical trials.

Microorganisms, 2021, 9(11), 2214., <https://doi.org/10.3390/microorganisms9112214>

[\[Tuberculosis\]](#)

A novel, rapid (within hours) culture-free diagnostic method for detecting live Mycobacterium tuberculosis with high sensitivity.

EBioMedicine, 2020, 60, 103007. <https://doi.org/10.1016/j.ebiom.2020.103007>

[\[Adiponectin\]](#)

Urinary adiponectin as a new diagnostic index for chronic kidney disease due to diabetic nephropathy.

BMJ Open Diabetes Research & Care, 2019, 7(1), e000661. <https://doi.org/10.1136/bmjdr-2019-000661>