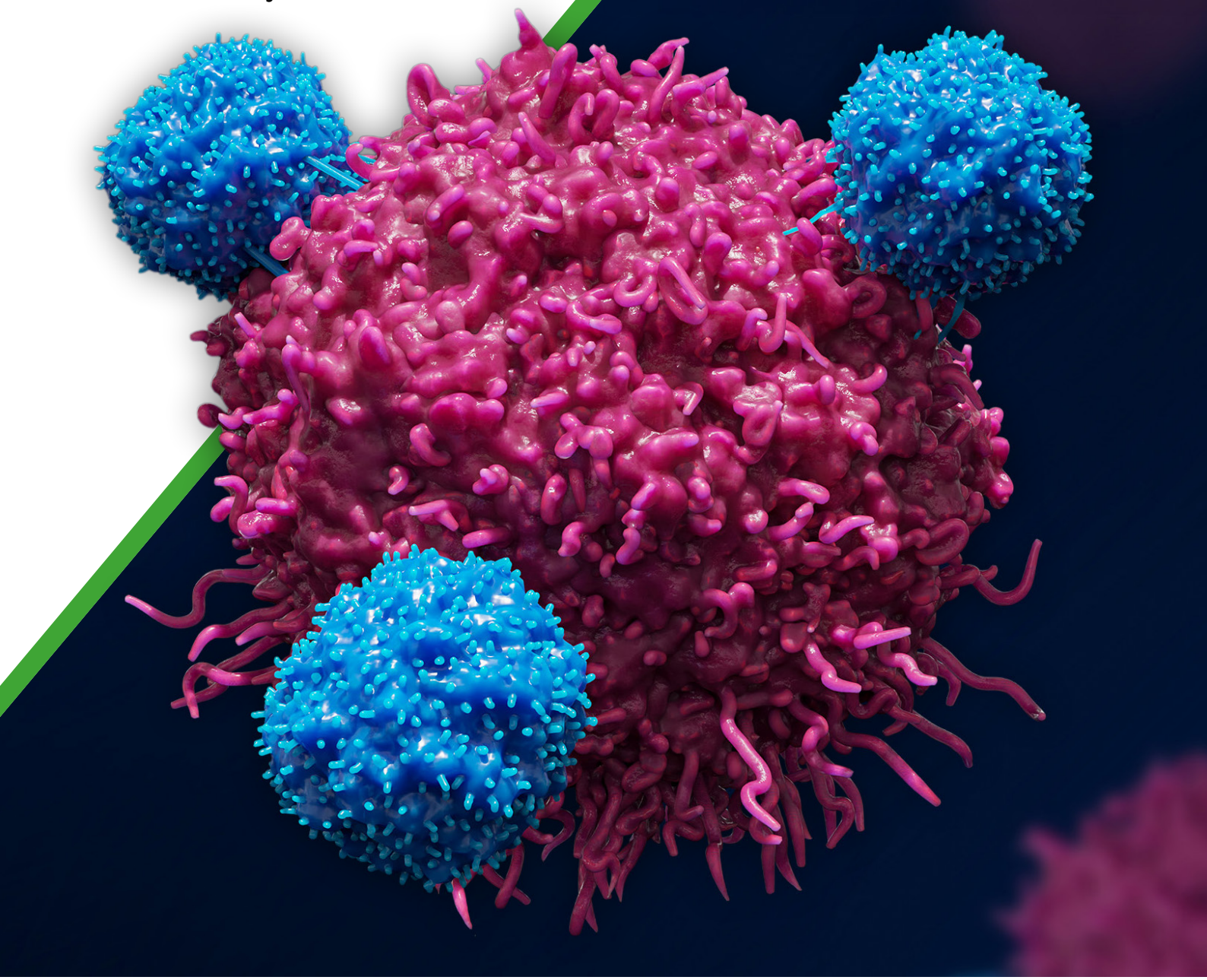
Early cancer detection with multiplex immunoassays

Your guide to achieving sensitivity, specificity, and scalability with multiplexed biomarker analysis



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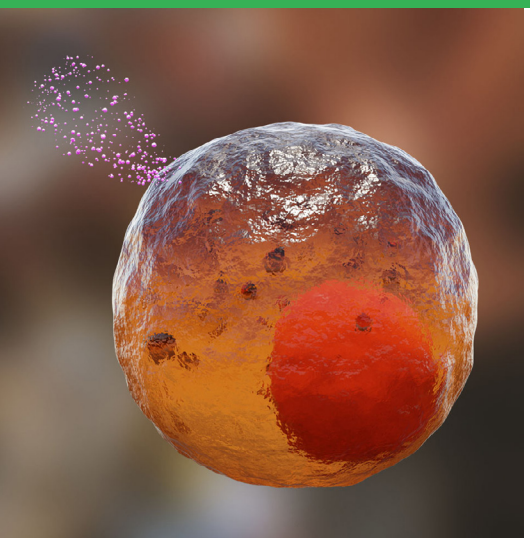
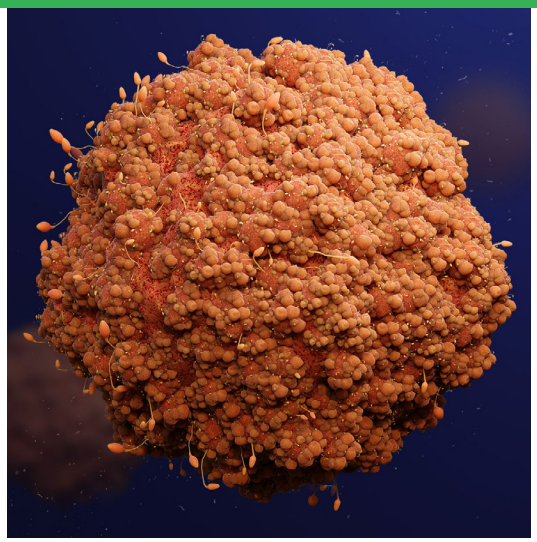
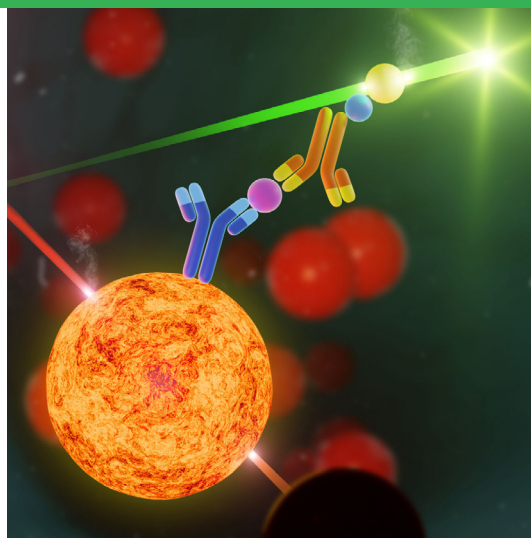

Introduction

Early cancer detection dramatically improves survival outcomes, yet many available diagnostic tools lack the sensitivity required to identify disease at its earliest— and most treatable—stages. Traditional single-analyte assays struggle to keep pace with the complex biology of tumor development, where networks of proteins, cytokines, and signaling molecules interact in ways that no single marker can fully represent.

Multiplex immunoassays offer a powerful alternative. By measuring dozens of biomarkers at once from minimal sample volumes, they provide the systems-level insights needed to detect subtle biological changes associated with early tumor formation. One example, R&D Systems™ Luminex® Assays, combines validated antibodies, optimized assay design, and flexible panel formats to accelerate biomarker discovery and support translational oncology research.

Contents

The challenge of effective early cancer detection

Despite major advances in oncology, early cancer detection remains difficult for several interconnected reasons. Sensitivity is an ongoing limitation, particularly for genomic-based methods, which struggle to detect low amounts of circulating tumor DNA in early-stage disease. At these stages, tumor burden is minimal, and biological signals are often faint or indistinguishable from background noise.

Compounding this issue is the inherent complexity of cancer biology. Tumors do not develop in isolation but through intricate, multifactorial networks that evolve over time. Single-biomarker approaches cannot adequately capture this complexity, making it difficult to generate a complete picture of early disease processes.

Matrix interference adds another layer of difficulty. Biological samples are crowded with high-abundance proteins, lipids, and antibodies that can mask or distort the analytes of interest, compromising accuracy. Even when these issues are addressed, reproducibility concerns persist, as variability in antibody quality, cross-reactivity, and

lot-to-lot performance can undermine longitudinal studies and multi-site research efforts. Finally, there is a significant gap between biomarker discovery and clinical implementation. Transitioning from research-use-only assays to validated laboratory developed tests requires extensive analytical validation, regulatory understanding, and scalable manufacturing – each a substantial hurdle that slows progress toward clinically actionable early detection tools.

The multiplex immunoassay solution

This guide contains a comprehensive collection of resources from R&D Systems, demonstrating how multiplex immunoassays can help overcome early cancer detection challenges – from real-world clinical success stories and strategic scientific rationale, through validated performance data and technical best practices, to achieving excellence in translational oncology research and diagnostic development.

Clinical success in bladder cancer detection

Bladder cancer provides a clear example of why improved early detection tools are urgently needed. Nearly one-third of patients are not diagnosed until stage two or later, when five-year survival rates fall to around fifty percent. Despite this clinical gap, no approved pre-symptomatic diagnostic exists, and traditional single-biomarker tests lack the sensitivity and specificity needed to meaningfully improve early detection.

This resource, demonstrates what is possible with a multiplex approach: the Oncuria® test. Developed using high-quality multiplex immunoassays from R&D Systems, this innovative panel measures ten bladder-cancer-associated proteins in urine samples. The ability to quantify all ten biomarkers in a single run – using validated antibodies and the sensitivity of R&D Systems multiplex assays – enabled clinical-grade performance for early detection, as well as risk stratification, therapy-response assessment, and non-invasive monitoring.

This success story provides a clear blueprint for advancing multiplex assays toward FDA Breakthrough Device Designation and LDT deployment.

The strategic advantage of proteomics

Leading cancer researchers are also turning to proteomics, integrating protein biomarkers with multiomics approaches and dramatically enhancing the sensitivity, specificity, and early intervention capabilities. Protein-level insights bring critical advantages over genomic approaches, particularly in early-stage disease where low-tumor burden is low and circulating DNA is often undetectable. In these scenarios, proteomics offers complementary – and sometimes earlier – signals, capturing dynamic biological activity that nucleic acids alone cannot reveal.

This whitepaper looks at the six core benefits of proteomics in early cancer detection, demonstrating how proteins complement nucleic acids in multiomics integration. Here, the R&D Systems multiplex assays successfully enable the simultaneous quantification of dozens of protein biomarkers needed to capture complex cancer biology and achieve the sensitivity advantages proteomics offers.

Multiplexed detection of tumor biomarker proteins

Consistent sample quality is essential for accurate tumor biomarker measurement, particularly in multi-site studies where variability in handling can introduce significant noise. Traditional collection tubes using EDTA or Heparin anticoagulants allow protein degradation and platelet activation during transport and storage. This subsequent variability in shipping and storage compromises biomarker measurements creating artifacts that obscure the true biological signals.

Validated data in this application note show how the 28-plex Human Tumor Biomarker Performance Panel from R&D Systems maintains excellent stability and reproducibility when paired with optimized sample collection methods. Using Streck plasma collection tubes helps preserves analyte integrity during the inevitable delays of transport and storage, ensuring that

biomarker measurements remain reliable. This compatibility provides the consistency and robustness needed for translating biomarker panels from discovery research to clinical diagnostics, where reproducibility across sites and over time is critical.

Meeting the demand for sensitivity, reliability, and efficiency

Cancer-derived samples – whether serum, plasma, urine, and tumor lysates – present unique analytical challenges. High-abundance proteins dominate these matrices, potentially overwhelming low-abundance targets. Lipids and other interfering substances can disrupt assay performance, while heterophilic antibodies introduce false signals that distort results. Together, these matrix effects can obscure true analyte signals, leading to false negatives, exaggerated positives, or compromised sensitivity that can distort results. Successfully developing multiplex immunoassays for clinical use means mastering these challenges, to ensure both clinical success and lot-to-lot reproducibility – a step particularly critical for longitudinal studies and commercial test development.

Beyond the technical considerations, successfully moving a multiplex assay from research use only (RUO) toward laboratory-developed test (LDT) status requires master of the regulatory and validation landscape. This guide explores how R&D Systems Luminex Assays combine rigorously validated antibodies, optimized diluents, and flexible formats to address every technical challenge from biomarker discovery to translation. With a menu of more than 450 targets, these solutions support biomarker discovery, verification, and translation, providing the scientific reliability and expert guidance needed to generate clinically meaningful, reproducible results at every stage of the research-to-clinic journey.

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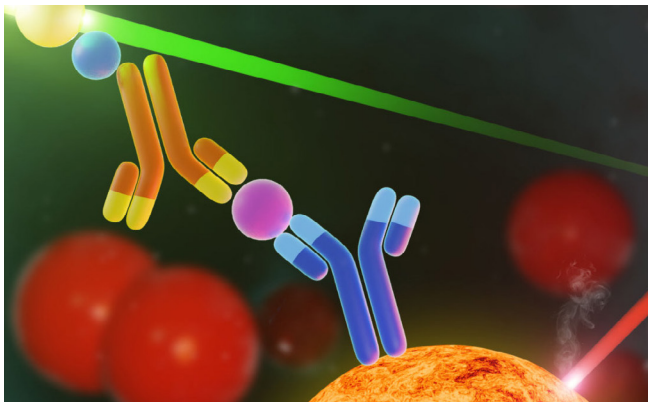
Solution Brief

Meeting the Challenges of Early Cancer Detection

Sensitivity Remains Elusive

Detecting cancers at their earliest stage increases the chance of long-term survival. While advances in genomic testing have led to progress in developing tools for early diagnosis, many current tests lack the sensitivity required to detect cancers early. One drawback to gene-expression analysis is that tumors shed cell-free DNA in a stage-dependent manner. In the early stages, when tumor burden is low, there is not much cell-free DNA entering the bloodstream. Moreover, that small amount of cell-free DNA has to be picked out of a higher baseline of other, non-tumor derived cell-free DNA[1].



Cancer Proteomics: the Key to Enhancing Sensitivity

Non-tumor derived signals from the tumor microenvironment are likely to be better indicators of cancer in earlier stages of cancer and more easily detectable. In recent years new proteomic-based technologies have enabled the identification of potential biomarkers that can be used to better detect cancers and tumor progression.

Additional studies have demonstrated the advantages of multiomics (the use of proteins along with nucleic acids) in increasing sensitivity. By adding proteomic algorithmic signature data, you get increased specificity and sensitivity, and increased probability of success for early detection[2].

FIGURE 1. CRC Sensitivity

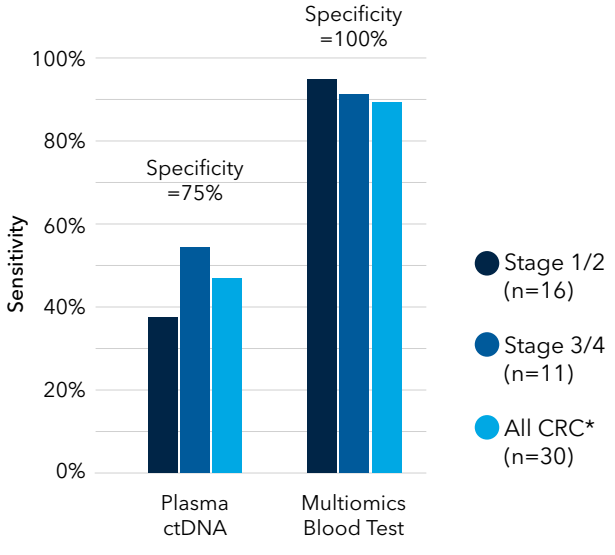


FIGURE 1. The multiomics blood test demonstrated 90% sensitivity and 100% specificity for CRC, whereas ctDNA achieved 47% sensitivity and 75% specificity.

How Are Proteomics Used to Detect Cancer Early and Accurately?

Bladder cancer is a good example. While it is not one of the most common types of cancer, bladder cancer has one of the highest rates of recurrence.

To date, there are no diagnostics capable of detecting bladder cancer prior to clinical presentation. Because of this severe limitation, nearly 30% of patients initially present with stage 2 and higher bladder cancer. Stage 2 bladder cancer has a 5-year survival rate of 50%[3].

That's where new multiplex protein assays are delivering promising results. Case in point is Oncuria®, a first-of-its-kind bladder cancer test from Nonagen Bioscience. Developed using high quality reagents from R&D Systems, this Luminex® multiplex panel detects concentrations of 10 proteins associated with bladder cancer in urine samples. Validated in more than 4,300 patients, Oncuria gives urologists a higher level of certainty compared to existing urine-based cancer and upper tract urothelial diagnostics.

In clinical studies, Oncuria was shown to have 93% sensitivity and 95% specificity for detecting bladder cancer.

FIGURE 2. Diagnostic Performance of Oncuria

	AUROC	Sensitivity	Specificity
Overall	0.95	0.93	0.93
Low-grade tumors	0.94	0.89	0.93
High-grade tumors	0.95	0.94	0.93
Low-grade tumors (NMIBC)	0.93	0.93	0.93
High-grade tumors (MIBC)	0.97	0.94	0.93

FIGURE 2. Diagnostic performance of Oncuria in parients with no personal bladder cancer history: presenting to a urology outpatient clinic for bladder cancer evaluation. 46 patients with de novo bladder cancer and 316 controls. Patients with no history of bladder cancer; n=362.

Charting a Path to LDT Development

To move robust biomarker panels closer toward clinical utility, research trials need sensitive and highly reproducible immunoassay systems that can simultaneously interrogate large numbers of targets per sample, in a short time.

These challenges underscore the importance of working with a trusted partner for assay RUO to LDT development. It's critical to look for a reliable supplier of raw materials with deep immunoassay development and manufacturing experience all conducted in a regulatory compliant environment.

The ability to design custom multiplex panels tailored to your unique needs helps save time, mitigate risk, and accelerate your path to LDT development. In a field defined by constant evolution, it's vital to equip yourself with the best tools to meet tomorrow's needs.

Learn more
bio-techne.com/reagents/luminex-assays/early-cancer-detection

Related Resources

1. Bredno J, Lipson J, Venn O, Aravanis AM, Jamshidi A. Clinical correlates of circulating cell-free DNA tumor fraction. PLoS One. 2021 Aug 25;16(8):e0256436.
2. Klein, Clinical Validation of a Targeted Methylation-based Multi-cancer Early Detection Test Using an Independent Validation Set. Ann Oncol, 2021 Sep;32(9):1167-1177
3. Charles J Rosser, MD, MBA, Hideki Furuya, Cedars-Sinai Medical Center, A Novel Multiplex Immunoassay for the Early Detection of Bladder Cancer, IIT2021-19-Furyua-EarlyDx, 2022-04-29

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White Paper

The Role of Proteomics in Early Cancer Detection: A Look at Six Core Benefits

Introduction

The advent of multi-omic approaches has improved early cancer detection by integrating diverse molecular data to gain a more complete understanding of early disease processes. While advances in genomic testing have led to progress in developing tools for early diagnosis, many current tests lack the sensitivity required to detect cancers early enough to improve long-term survival.

In recent years, new proteomic-based technologies have enabled the identification of potential biomarkers that can be used to better detect cancers and tumor progression. To move robust biomarker panels closer toward clinical utility, however, research trials need sensitive and highly reproducible immunoassay systems that can simultaneously interrogate large numbers of targets per sample in a short time. That's where Luminex's xMAP® Technology has emerged as a valuable tool. Incorporating multiple non-tumor biomarkers on the xMAP platform into a multiomic early cancer detection assay can potentially enhance sensitivity, specificity, and accuracy, improving early detection and leading to earlier intervention and better patient outcomes.

Here are six key benefits of integrating protein biomarkers into the development of early cancer detection assays:

1. Protein Patterns

As cancer progresses quantitative profiling of numerous proteins and monitoring the patterns of protein expression over time can assist in identifying biomarkers that effectively track disease progression and the response to therapy. These biomarkers can potentially detect the patient's immune response to early cancer processes, even before any cancer-specific biomarker is detectable in the bloodstream. By focusing on the immune response, which can occur prior to the release of traditional cancer biomarkers, we can potentially detect and intervene in cancer at an earlier stage.[3,6,7]

2. High Sensitivity

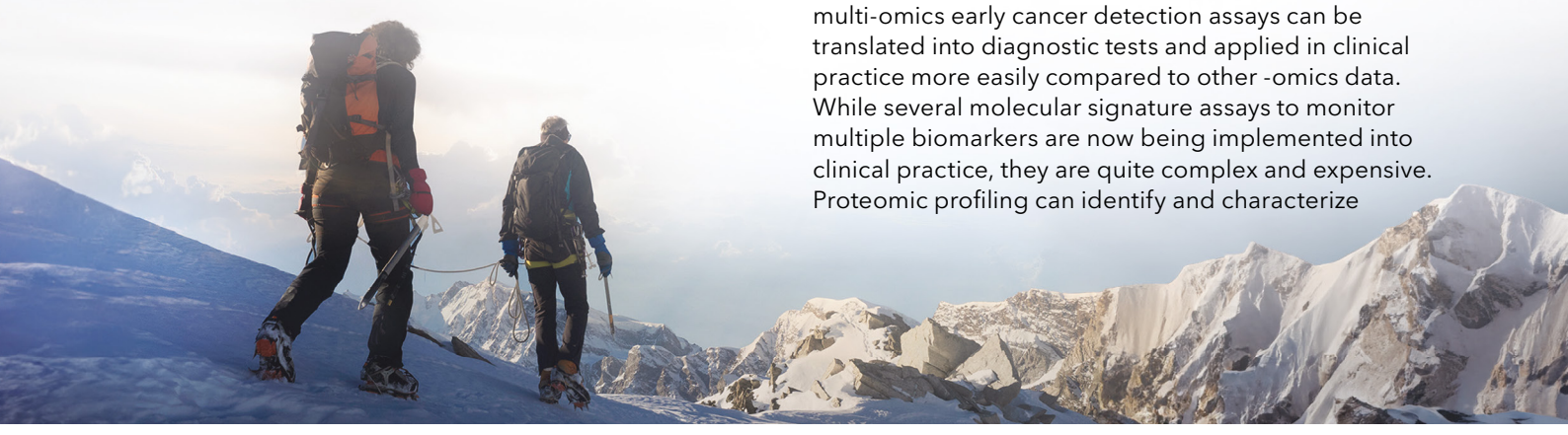
Recent studies have demonstrated the increased sensitivity of adding proteomic algorithmic signature data to multi-cancer early detection studies. Proteomics techniques allow for the detection of minute changes in protein expression levels that can occur during the early stages of cancer development. These changes may not be easily detectable through other diagnostic methods. Proteomic analysis can help identify specific protein biomarkers associated with different types of cancer, providing highly sensitive detection and potential early warning signs of the disease.[6]

3. Non-invasive Sampling

Many protein biomarkers can be detected non-invasively through bodily fluids like blood, urine, or saliva. This allows for convenient and relatively non-invasive sample collection, making it feasible to perform regular screenings and monitor high-risk individuals more effectively. Non-invasive tests also reduce patient discomfort and increase compliance with screening programs, allowing for more frequent monitoring and early cancer detection.

4. Practical Implementation

Protein biomarkers that show promising results in multi-omics early cancer detection assays can be translated into diagnostic tests and applied in clinical practice more easily compared to other -omics data. While several molecular signature assays to monitor multiple biomarkers are now being implemented into clinical practice, they are quite complex and expensive. Proteomic profiling can identify and characterize



proteins that are indicative of early-stage cancer and can readily serve as targets for diagnostic tests or potential therapeutic interventions. Incorporating protein biomarkers into multi-omics assays increases the potential for practical implementation in routine clinical settings.[1,3,6,7]

5. Early Intervention

Recent advances in proteomic-based technologies have led to progress in developing tools for early diagnosis, including valuable prognostic and predictive information. Certain protein signatures can help predict the likelihood of disease progression, response to specific treatments, or even the risk of recurrence after initial treatment.

Protein biomarkers and tumor-derived nucleic acids complement each other in early cancer detection, wherein nucleic acid-based tests detect specific genetic alterations, and protein biomarkers reveal functional consequences such as changes in pathways, interactions, and modifications. The integration of both protein and nucleic acid biomarkers enhances the sensitivity, specificity, and accuracy of multi-omics approaches, which can guide personalized treatment decisions and enable early intervention strategies.[3,4,5,6]

6. Protein Abundance

Protein biomarkers can be derived from multiple sources, including tumor cells, tumor microenvironment, or other tissues affected by cancer. When released into the bloodstream or other bodily fluids, these protein biomarkers provide a broader range of potential targets for early cancer detection. In contrast, tumor-derived nucleic acids are specifically released by cancer cells and carry genetic alterations specific to the tumor. While they provide direct information about the presence of cancer-related mutations, their limited abundance and potential dilution in non-tumor-derived genetic material make their detection more challenging.[3,4,5,6]

Gaining an Edge in Early Cancer Detection

Both protein biomarkers and tumor-derived nucleic acids offer valuable information in early cancer detection, and their integration in multi-omics approaches can provide a more comprehensive understanding of cancer biology and help make diagnostic testing more accurate and actionable. This is precisely the overarching goal of the partnership between Luminex Corporation and R&D Systems.

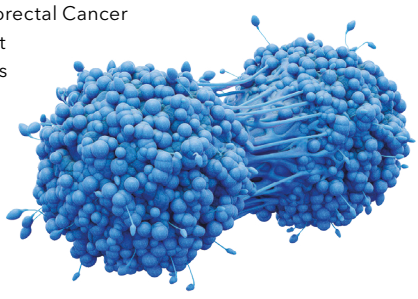
Luminex has established a solid reputation for providing high-quality multiplex assay systems to research and clinical diagnostic laboratories worldwide, so commercializing an xMAP assay through Luminex means associating with a reputable company with a proven track record in the industry.

Furthermore, R&D Systems' Luminex-based multiplex assays combine high-quality reagents and over 30 years of industry-leading immunoassay experience to create a powerful solution to advance early cancer detection strategies.

This innovative partnership combines the world's leading antibody, protein, and ELISA manufacturer with the world's leading platform for multiplex proteomics—with the joint mission to accelerate the development and commercialization of early cancer detection.

References

1. AACR 2022: Single-Cell, Spatial, and Early Detection Rising to New Heights April 14, 2022 Cowen.
2. A New Era of Protein-Based Assays for Cancer Early Detection, Journal of Thoracic Oncology Vol. 16 No. 2 Lecia V. Sequist, MD, MPH,* Steven J. Skates, PhD, Wilhelm Haas, PhD https://www.jto.org/action/showPdf?pii=S1556-0864%2820%2931095-9.
3. Biomarkers for Early Detection of Colorectal Cancer: The Early Detection Research Network, a Framework for Clinical Translation. Robert S. Bresalier, William M. Grady, Sanford D. Markowitz, Hans Jørgen Nielsen, Surinder K. Batra, and Paul D. Lampe watermark. silverchair.com/2431.pdf?token=AQECAHi208BE49Ooan9kkhW_.
4. Multianalyte Tests for the Early Detection of Cancer: Speedbumps and Barriers. Michael A. Tainsky, Madhumita Chatterjee, Nancy K. Levin, Sorin Draghici and Judith Abrams
5. Proteomics for early detection of colorectal cancer: recent updates https://www.tandfonline.com/doi/abs/10.1080/14789450.2018.1396893?journalCode=ieru20.
6. Blood-based detection of early-stage colorectal cancer using multiomics and machine learning. Meeting Abstract | 2020 Gastrointestinal Cancers Symposium Girish Putcha, Tzu-Yu Liu, Eric Ariazi, Marvin Bertin, Adam Drake, Michael Dzamba https://ascopubs.org/doi/abs/10.1200/JCO.2020.38.4_suppl.
7. Multi-Omics Approaches in Colorectal Cancer Screening and Diagnosis, Recent Updates and Future Perspectives Ihsan Ullah, Le Yang, Feng-Ting Yin, Ye Sun, Xing-Hua Li, Jing Li and Xi-Jun Wang,* https://www.mdpi.com/2072-6694/14/22/5545.



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Luminex Assays
Application Note

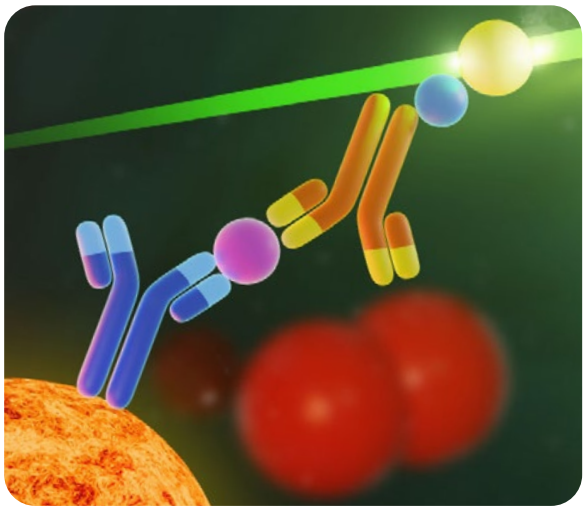
Multiplexed Detection of Tumor Biomarker Proteins

Using Luminex Assays and Streck Plasma

Introduction

Luminex® High Performance assays from R&D Systems™, a Bio-Techne brand, offer a powerful tool for the multiplexed detection of high-importance biomarkers while providing high sensitivity and industry-leading precision and reproducibility. With the newly released Human Tumor Biomarker Performance Panel, a 28-plex immunoassay measuring soluble tumor biomarkers, these Luminex assays for the first time have been validated for use with plasma samples collected with **Streck® Cell-Free DNA BCT®** or **Streck Protein Plus BCT™**. Leveraging advanced Luminex xMAP® technology, this powerful solution includes a comprehensive array of biomarkers pivotal in understanding tumor biology, progression, and response to therapy. By incorporating a wide spectrum of tumor markers, including those associated with liver cancer, ovarian cancer, breast cancer, pancreatic cancer, neuroendocrine tumors, and thyroid cancer, it supports comprehensive profiling and fosters a deeper understanding of tumorigenesis and metastasis. Each biomarker has been validated to ensure optimal performance. The combination of these high-quality assays and sample types will improve the integration of genomic and proteomic tools for multicancer early detection research and diagnostic development.

Streck Cell-Free DNA BCT contains preservatives that maintain cell-free DNA and limit the release of genomic DNA, minimizing the degradation of circulating tumor cells that are crucial to many downstream research and development applications. The Streck Protein Plus BCT contain preservatives designed to minimize *ex vivo* hemolysis and platelet activation in the collected sample, limiting interference from proteins released post-draw that can add error to the true abundance of the circulating analytes of interest. The stabilizing effects in the Streck tubes allows for the collection of samples from multiple sites while utilizing a single site for sample processing and analysis. The added stability time means researchers can ship samples without worrying about lapses in cold chain storage, or about differences in sample handling at the collection sites.



Methodology

The stability of 28 multiplexed analytes was evaluated using the Human Tumor Biomarker Performance Panel (Catalog # **FCSTM25**). Samples from 20 presumed healthy individuals were collected in EDTA, Heparin, Streck Cell Free DNA BCT, and Streck Protein Plus BCT. Plasma was isolated and processed per the manufacturer's guidelines after 0, 3, or 5 days post-draw at room temperature. Aliquots of processed samples were stored at -20 °C until time of evaluation.

The EDTA and Heparin samples were diluted 2-fold with assay diluent, while the Streck BCT and Protein Plus samples were diluted either 2-fold or 8-fold with assay diluent. Four sets of assays were performed on separate days. Reported concentrations were back-calculated for the appropriate dilution factor.

Results

The Streck Cell Free DNA BCT and Protein Plus BCT demonstrated superior stability for the analytes in the Human Tumor Biomarker Panel compared to EDTA and heparin tubes. As shown in Figure 1, limited analytes had sample stability observed across all tube types. Figure 2 highlights the consistent performance of multiple analytes in the Cell Free DNA and Protein Plus BCT tubes, while the EDTA and heparin tubes exhibited variability at days 3 and 5.

Additionally, Figure 3 shows that both Streck tubes performed exceptionally well with IL-8, which forms heterodimers with platelet factor 4 (PF4), a protein released at high concentrations from platelets. IL-8 concentrations were notably elevated in EDTA and heparin plasma samples at days 3 and 5.

FIGURE // 01

Analyte Stability

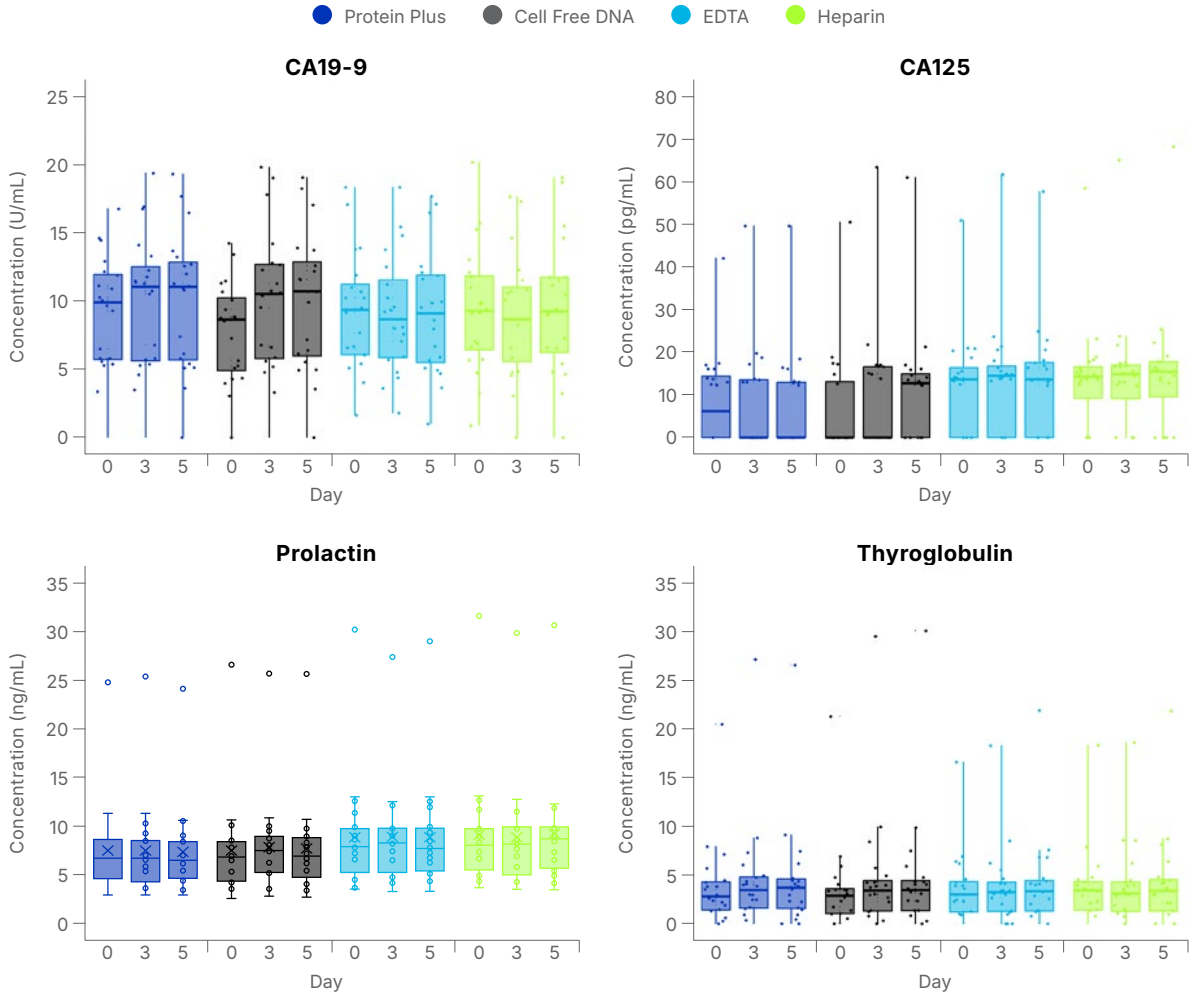


Figure 01. CA19-9, CA125, Prolactin, and Thyroglobulin sample levels remain consistent across all tubes and days.

FIGURE // 02

Performance in Protein Plus BCT Tubes

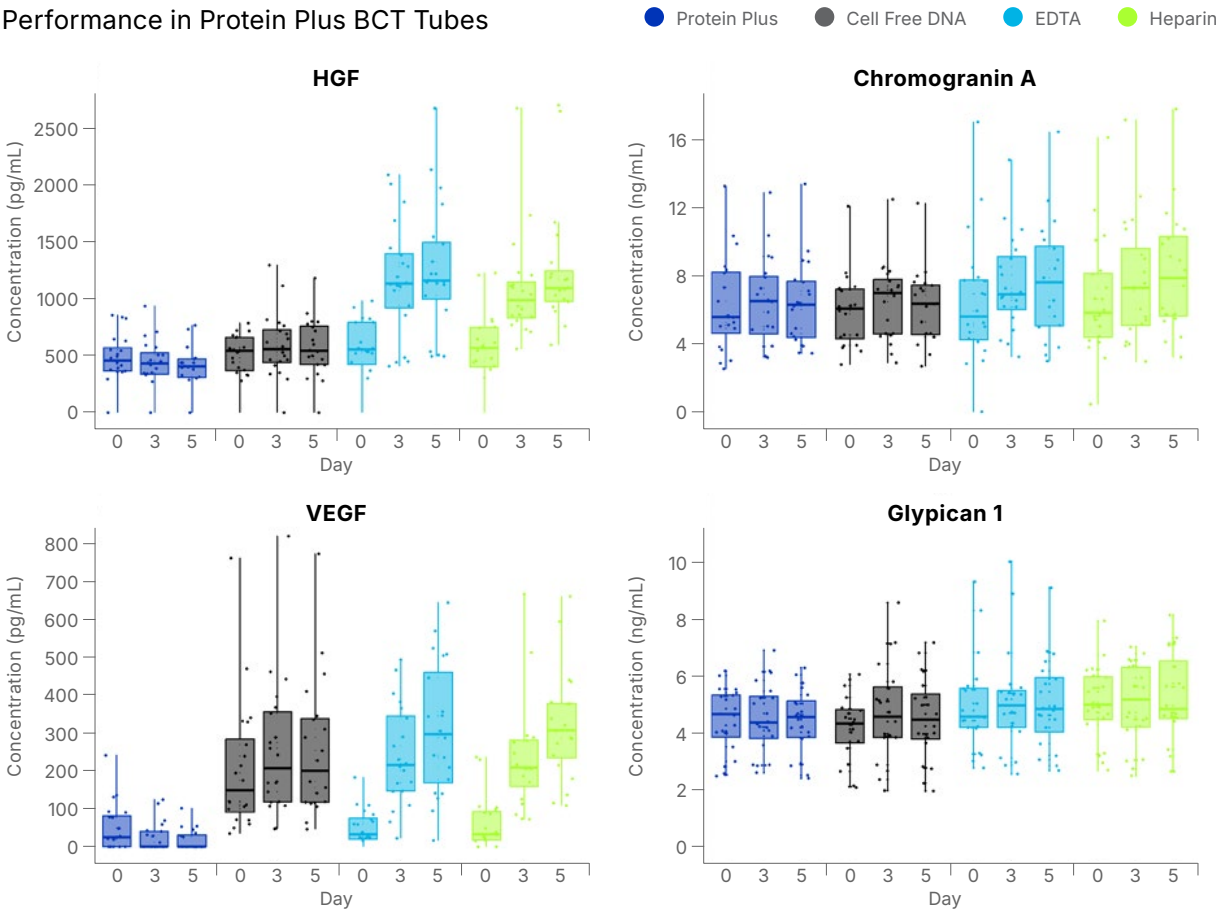


Figure 02. Sample values remained stable in Streck Cell Free DNA BCT and Protein Plus tubes while sample values increased over time in EDTA and heparin.

FIGURE // 03

Stability Over Time

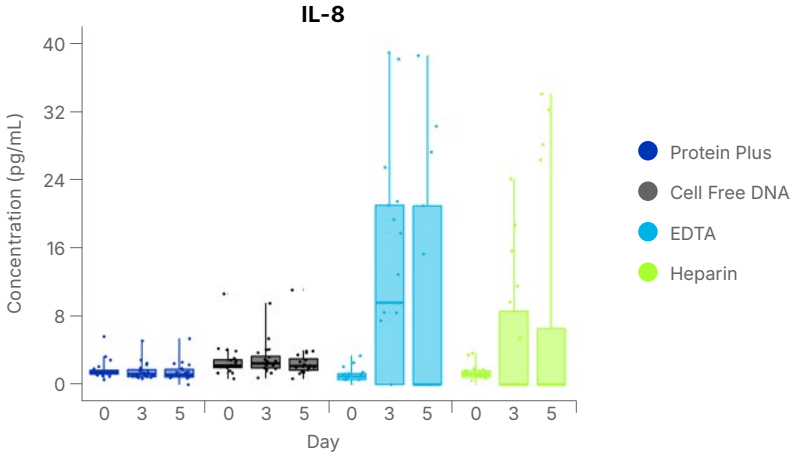


Figure 03. IL-8 is known to form heterodimers with PF4. In both Streck Cell Free DNA BCT and Protein Plus tubes levels remained stable over time while both EDTA and heparin IL-8 levels continued to increase over time.

Summary

The combination of Streck plasma tubes and Bio-Techne's Human Tumor Biomarker Performance Panel are a winning combination for stability and sensitivity in the discovery and development of soluble tumor biomarkers associated with cancer. The Streck tubes stabilized most of these important tumor biomarker analytes when stored at room temperature prior to processing and evaluation. The Protein Plus tubes were the best at stabilizing analytes with known stability issues related to platelet activation and release (including CD40 ligand, IL-8, and MIF).

Streck Cell-Free DNA BCT outperformed Protein Plus BCT for ENO-2 and MIF stability. The tube type and collection procedure can have an impact on the stability of some analytes. It is important to test analyte stability early in studies with large cohorts. Any large study should involve the validation of possible sample handling differences across sites and could follow a similar study plan to what is presented here.

Conclusion

The integration of the Human Tumor Biomarker Luminex Performance Panel with Streck® Cell-Free DNA BCT and Protein Plus BCT offers a robust solution for multiplexed detection of tumor biomarkers, ensuring high stability and accuracy in plasma samples even under varied conditions. Our findings demonstrate that these tubes effectively preserve the integrity of critical analytes, with the cfDNA BCT showing superior stability for certain platelet-sensitive markers like MIF and IL-8. Protein Plus BCT also excels in stabilizing analytes prone to degradation due to platelet activation. These results underscore the importance of selecting the appropriate sample collection and handling methods in large-scale cancer studies, as they directly impact the reliability of biomarker detection. This combined approach of advanced assay technology and optimized sample preservation stands to significantly enhance the precision and reproducibility of multicancer biomarker research and diagnostics.

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Multiplex Immunoassays
Technical Note

The Role of Multiplex Immunoassays in Early Cancer Detection

Meeting the Demand for Sensitivity, Reliability, and Efficiency

Nearly 30% of bladder cancer patients are diagnosed at stage 2 or higher, where five-year survival falls to around 50%.[1] The reason? Current diagnostics cannot reliably detect the disease before clinical presentation. This gap represents more than a technical challenge—it reflects a fundamental limitation of single-biomarker approaches in a disease defined by complexity.

Cancer progression and therapeutic response rarely depend on a single molecular signal. Instead, they reflect the interplay of cytokines, growth factors, and signaling networks within the tumor microenvironment. To capture this complexity, researchers are increasingly turning to multiplex immunoassays—platforms capable of measuring dozens of analytes from small-volume samples.

By conserving patient specimens, increasing throughput, and revealing systems-level insights, multiplexing provides a more comprehensive view of tumor biology than single-analyte approaches can offer. But realizing this potential demands assays that deliver more than high throughput. They must achieve ultra-high sensitivity, reproducibility, and low cross-reactivity, while performing reliably in complex matrices such as serum or plasma.

Meeting these standards requires antibody expertise, rigorous validation, and panel designs flexible enough to address diverse research needs. **R&D Systems Luminex® Assays Instruments** combine broad multiplexing capacity with validated antibodies, optimized diluents, and one of the industry's largest mix-and-match analyte menus, providing the sensitivity, reliability, and flexibility essential for advancing cancer detection and therapeutic development.

Connecting the Dots in Cancer Biology

Cancer biology cannot be reduced to a single molecular signal. Cytokines such as IL-6, TNF-α, and IL-10 play overlapping yet distinct roles in the tumor microenvironment. Measuring one analyte provides only a fragment of the picture, whereas multiplexing offers a panoramic view of immune–tumor interactions. This broader perspective enables the identification of biomarker patterns that correlate with disease stage, predict therapeutic response, or signal recurrence.

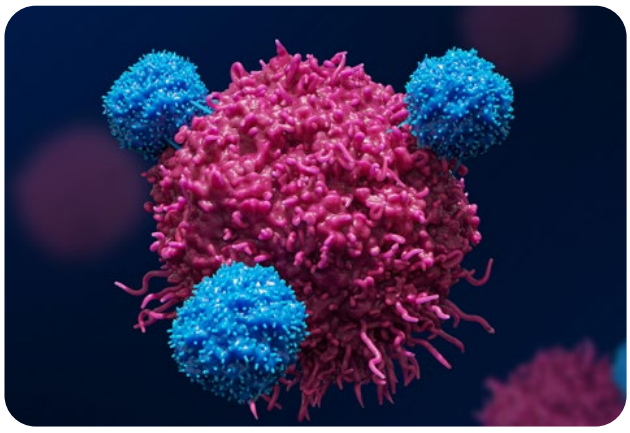
Traditional single-analyte assays fall short in this setting. They may overlook subtle but clinically important changes in low-abundance cytokines or fail to capture the interplay between opposing immune factors. For instance, a rise in a pro-inflammatory cytokine could be counterbalanced by an anti-inflammatory mediator—a dynamic invisible when measured in isolation.

Multiplex immunoassays overcome these limitations by simultaneously quantifying dozens of cytokines and soluble factors from a single plasma or serum sample. This dual capture of tumor-derived signals and systemic immune activation has already revealed cytokine signatures linked to prognosis and treatment response. In studies of gastrointestinal cancers, patients with high baseline levels of two cytokine patterns—one associated with checkpoint activity and another with T-cell trafficking—showed better prognosis and higher overall response rates to checkpoint blockade. Critically, multiplex profiling enabled these signatures to be identified before clinical outcomes were evident.[2]

Longitudinal multiplex analysis extends this capability further, tracking immune shifts during therapy and distinguishing emerging responders from those developing resistance or toxicity. Such insights not only refine patient selection but also give clinicians the opportunity to adapt treatment strategies earlier and more effectively.

Accuracy Starts with the Right Antibodies

Detecting low-abundance biomarkers—such as early-stage tumor cytokines—requires assays with both exceptional sensitivity and specificity. In multiplex settings, this challenge is even greater: the assay must remain sensitive enough to detect subtle changes while avoiding cross-reactivity among closely related proteins.



The key lies in antibody quality. High-performance systems rely on rigorously selected antibody pairs with proven specificity. Extensive validation against potential cross-reactivity ensures that even large, complex panels produce accurate, reproducible results. This attention to antibody design allows multiplex assays to detect analytes at picogram-per-milliliter concentrations—levels that can serve as early warning signs before clinical symptoms emerge.

Recent advances in multiplex technology have pushed sensitivity into new territory. Modern platforms such as the **Luminex xMAP® System** can now achieve detection limits as low as 0.06 pg/mL. Widely applied for protein assays—including cytokines, chemokines, and growth factors—as well as for gene expression analysis, Luminex technology exemplifies how technical innovation directly expands research capability.

These advances translate into meaningful clinical insight. In a lung cancer biomarker study, researchers used an **R&D Systems Human XL Cytokine Luminex® Performance Assay** to measure both VEGF and PD-L1 in patient serum.[3] The panel delivered ELISA-comparable sensitivity while enabling simultaneous detection, revealing co-expression patterns linked to tumor angiogenesis and immune evasion. This dual readout provided a more complete picture of pathway interactions than single-biomarker approaches could achieve.

By analyzing VEGF and PD-L1 together, the study identified prognostic indicators of poor survival in lung adenocarcinoma while highlighting the therapeutic potential of targeting angiogenesis and immune evasion in tandem. Multiplex profiling exposed complex biological signatures within the tumor microenvironment that would remain invisible in isolation—underscoring how integrated biomarker analysis sharpens both prognostic insight and treatment strategy.

Turning Complexity into Clarity

Cancer samples such as serum, plasma, urine, and tumor lysates present a uniquely difficult testing environment. They are rich in high-abundance proteins, interfering lipids, and heterophilic antibodies—factors collectively known as matrix effects. Left unaddressed, these interferences can mask true analyte signals, leading to false negatives, exaggerated positives, or loss of sensitivity.

One of the most common challenges lies in the imbalance between high- and low-abundance proteins. For example, IL-8 may be present at very high levels while IL-17 exists only in trace amounts. Without careful dilution and assay design, the stronger IL-8 signal can overwhelm IL-17, making accurate detection nearly impossible.

R&D Systems Multiplex Assays are engineered to overcome these barriers. Optimized diluents and buffers are specifically formulated to minimize nonspecific binding, reduce cross-reactivity, and control variability from factors such as pH, viscosity, or salt concentration. This ensures accurate spike recovery and linear dilution across a wide range of analytes—even in the most complex biological samples. Signal integrity is preserved for both high- and low-concentration proteins, delivering enhanced precision and lot-to-lot reliability across panels measuring dozens of biomarkers.

Adapting Panels for Discovery and Validation

Cancer is not a static disease. As tumors evolve, so too does the need to monitor shifting patterns of protein expression. Because no two cancer studies are alike, assay flexibility is essential. R&D Systems addresses this need with two complementary formats:

- Discovery Assays offer customizable panels with access to more than 450 analytes, ideal for broad exploratory research where researchers cast a wide net to identify candidate biomarkers.
- High Performance Assays provide pre-validated panels optimized for sensitivity and reproducibility on par with ELISA, designed for translational and clinical applications where refinement and validation are critical.

This two-tiered model provides a natural progression: researchers begin with Discovery Panels to explore the biological landscape, then transition to High Performance Panels for focused validation as studies advance toward clinical endpoints.

Building on this foundation, R&D Systems recently introduced the Human Tumor Biomarker Performance Panel—a rigorously validated 28-plex assay optimized for plasma samples collected in preservative blood collection tubes. Leveraging Luminex xMAP technology, the panel encompasses tumor markers relevant to liver, ovarian, breast, pancreatic, neuroendocrine, and thyroid cancers. Each analyte has undergone stringent validation to ensure optimal performance in plasma-based applications.

Pairing validated multiplex panels with specialized sample collection methods further strengthens early detection workflows. Preservative tubes such as Streck® Cell-Free DNA BCT® and Protein Plus BCT™ maintain sample integrity by preventing genomic DNA release and preserving circulating tumor components critical for downstream analysis. Together, these advances bridge genomic and proteomic tools—bringing multi-cancer early detection and diagnostic development within closer reach.

From Research to Clinical Impact: The Oncuria Story

The path from biomarker discovery to clinical diagnostics is notoriously difficult. It requires not only scientific insight but also platforms that combine sensitivity, reproducibility, and validated reagents capable of meeting regulatory standards. Bladder cancer diagnostics illustrate both the challenge and the opportunity.

Although relatively uncommon, bladder cancer has one of the highest recurrence rates among cancers. Current diagnostics are unable to reliably detect the disease before clinical presentation; nearly 30% of patients are first diagnosed at stage 2 or higher, where the five-year survival rate falls to around 50%. The clinical need for earlier, more accurate detection is clear.

To address this gap, Nonagen Bioscience developed Oncuria®, a first-of-its-kind multiplex protein test built with high-quality reagents from R&D Systems. Oncuria measures 10 proteins associated with bladder cancer in urine samples and has been validated in more than 4,300 patients. Clinical studies demonstrated 93% sensitivity and 95% specificity—giving urologists greater diagnostic confidence than existing urine-based methods.[4] (See Figure 1)

FIGURE 1.

	AUROC	Sensitivity	Specificity
Overall	**0.95**	**0.93**	**0.93**
Low-grade tumors	0.94	0.89	0.93
High-grade tumors	0.95	0.94	0.93
Low-grade tumors (NMIBC)	0.93	0.93	0.93
High-grade tumors (MIBC)	0.97	0.94	0.93

Figure 1. Diagnostic performance of Oncuria in parients with no personal bladder cancer history: presenting to a urology outpatient clinic for bladder cancer evaluation. 46 patients with de novo bladder cancer and 316 controls. Patients with no history of bladder cancer; n=362.

Diagnostic Performance of Oncuria

This case underscores a broader reality: advancing from research use only (RUO) assays to laboratory-developed tests (LDTs) requires platforms that combine sensitivity, reproducibility, and validated reagents. Cancer research often spans years, which makes reproducibility across assay lots absolutely critical. Even small inconsistencies can compromise longitudinal data, undermining confidence in insights related to disease progression, relapse, or therapeutic response.

To ensure consistency, every R&D Systems assay panel undergoes rigorous validation—particularly important in longitudinal oncology trials, such as tracking cytokine release in patients undergoing immunotherapy. Because these studies often rely on limited clinical specimens, sample conservation is essential. Multiplex assays meet both requirements, enabling broad biomarker profiling from as little as 25–50 µL of serum or plasma, making repeated measurements possible even in constrained patient cohorts.

Trusted suppliers with deep immunoassay expertise and regulatory-grade manufacturing serve as essential partners in this process. Custom panel design further accelerates the RUO-to-LDT pathway, enabling researchers to save time, mitigate risk, and build assays capable of meeting clinical standards. The Oncuria example demonstrates how multiplex assays can transition from research tools to clinically actionable diagnostics—transforming biomarker discovery into real-world diagnostic impact.

The Path Forward: Integration and Precision

One of the most promising aspects of protein biomarkers is their detectability in minimally invasive samples such as blood, urine, or saliva. This opens the door to routine monitoring and population-wide screening with far greater patient comfort and compliance. By reducing barriers to participation, non-invasive sampling makes it possible to follow high-risk individuals more closely and detect disease earlier.

Multiplex immunoassays extend this advantage by enabling sensitive, simultaneous measurement of multiple biomarkers from very small sample volumes. Dozens of analytes can be quantified in just hours using standard laboratory instrumentation—work that would otherwise take days if run as single-analyte assays. The result is faster data generation, streamlined workflows, and more timely decision-making in both clinical trials and translational research.

Early cancer detection demands more than a single molecular signal. Protein biomarkers and tumor-derived nucleic acids each provide valuable insights, but together—integrated within multi-omics approaches—they deliver a more complete view of cancer biology. Multiplex immunoassays sit at the center of this evolution, enabling systems-level analysis that supports earlier detection, prediction of therapeutic response, and personalized treatment strategies.

The hurdles of multiplexing—cross-reactivity, matrix effects, dilution challenges, and reproducibility—have always been part of the equation. Advances in antibody quality, assay design, and validation now make it possible to generate reliable, reproducible data even from complex clinical samples. With high sensitivity, broad analyte coverage, flexible panel formats, and decades of immunoassay expertise, R&D Systems Assays provide researchers with tools that are both rigorous and practical.

As cancer research enters a new era defined by precision and integration, multiplex immunoassays provide more than just technical solutions—they lay the foundation for earlier detection, more effective therapies, and ultimately, better patient outcomes.

Key Considerations When Selecting Multiplex Assays

- **Antibody validation:** Build on well-characterized antibodies tested for cross-reactivity and proven for multiplex performance.
- **Panel-wide validation:** Choose panels validated as integrated systems, not just collections of single-analyte assays.
- **Matrix optimization:** Account for high- and low-abundance targets with proper grouping and optimized diluents.
- **Supplier expertise:** Partner with suppliers who combine antibody expertise and assay development experience to reduce risk and ensure reliable results.

R&D Systems Luminex Assay Capabilities

- **Flexible formats:** Discovery and High Performance Panels designed for different research stages.
- **Validated quality:** Antibodies tested for specificity, panels validated for recombinant and natural proteins.
- **Efficient workflows:** Generate results in 3–3.5 hours using ≤50 µL of sample.
- **Proven reproducibility:** Stringent lot-to-lot QC ensures consistent performance over time.

REFERENCES

1. Charles J Rosser, MD, MBA, Hideki Furuya, Cedars-Sinai Medical Center, A Novel Multiplex Immunoassay for the Early Detection of Bladder Cancer, IIT2021-19-Furyua-EarlyDx, 2022 04-29
2. Yarahmadi A, Afkhami H. The role of microbiomes in gastrointestinal cancers: new insights. Front Oncol. 2024 Feb 1;13:1344328. doi: 10.3389/fonc.2023.1344328.
3. Journal, Cancer Research, Publisher: American Association for Cancer Research. PMID: 37074042; PMC Id: 10236157; DOI: 10.1158/0008-5472.CAN-21-4425
4. Furuya H, Tabula L, Lee R, Kralovec P, Ramsden M, Wong R, Rosser CJ. Analytical validation of ONCURIA™ a multiplex bead-based immunoassay for the non-invasive bladder cancer detection. Pract Lab Med. 2020 Nov 13;22:e00189. doi: 10.1016/j.plabm.2020.e00189.

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