Characterized Biospecimens:

A Guide for Leveraging Deeply Characterized Biospecimens to Support Successful Therapeutic & Diagnostic Development

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Table of Contents

Introduction

Biospecimens are critical for advancing basic science, translational research, and clinical trials. Used for diverse applications ranging from illuminating the mechanisms of disease to identifying those patients most likely to respond to specific drugs, biospecimens play an integral function in advancing the development of targeted therapies and precision medicine. To realize the full potential of their value, biospecimens must be sourced and collected appropriately and ethically and then characterized and paired with relevant clinical information to generate actionable data.

Why are characterized specimens crucial for successful development?

A deep understanding of the disease of interest is fundamental to any drug development effort. For researchers, the challenge lies in elucidating the mechanistic biology of a disease and identifying targetable drivers. Biospecimens play a crucial role in this phase of discovery by enabling comparison of the target patient population to controls and bridging molecular insights and clinical information. Here, it is important to keep in mind that the data derived from biospecimens is only as good as the samples and not all samples are created equal. To optimize target identification, drug development, and patient selection, it is essential to have the right biospecimens—fit-for-purpose samples that are well characterized and that can be used to answer the scientific question being asked. With characterized biospecimens, it is possible to put molecular findings into the appropriate clinical context, thus ensuring accurate interpretation of study results and accelerating development timelines.

Key considerations for sourcing biospecimens

High-quality, compliant biospecimens are critical throughout the development continuum. To ensure that biospecimens are fit for their intended purposes, researchers should consider the following factors, all of which can affect data quality and reliability:

- **Specimen quality**. The integrity of a sample can be compromised by freeze-thaw cycles, time elapsed since collection, and improper fixation, storage, transport, or chain of custody.
- **Specimen availability**. Large cohorts are needed to confirm if a discovery is valid across most patients with the disease of interest. In addition, harmonized clinical and test data will be needed across a sizable enough cohort to support analysis.
- **Informed consent**. Specimens must be consented appropriately for their intended use.
- **Specimen types needed**. It is imperative for researchers to understand and identify the specimen types necessary for answering their scientific question. They will typically need sample matrices that can be used to analyze or measure disease biology, matched controls to confirm potential drug targets are not present in healthy subjects, and matched specimen types (eg, serum, tumor tissue, and DNA from the same patient).

Biospecimen characterization enables researchers to extract maximum value out of precious samples. Ideally, samples are deeply phenotyped, with accompanying data on disease severity, comorbid conditions, past treatment response, and other relevant clinical history.

The importance of high-quality biospecimens in development

❶ Reagent validation

Validation of key reagents, such as reference standards, proteins, antibodies, labeled analytes, and detector reagents, is essential for ensuring the integrity and suitability of an assay for its intended use. It is also important to verify that assay results are consistent among different reagent lots and across instrument platforms. Ideally, this validation and verification are performed on a large quantity of well-characterized biospecimens that are representative of the target demographic or disease state.

For companion diagnostic (CDx) validation, the FDA frequently requires orthogonal testing, which involves utilizing multiple independent assays or testing methods to confirm results. FDA guidelines typically stipulate that the orthogonal methods selected should differ fundamentally from the CDx. For example, if the CDx is an immunoassay, the orthogonal test could be a different type of immunoassay, a molecular method, or another appropriate technology. This approach aims to mitigate risks associated with false positives or negatives, thereby enhancing the reliability and robustness of the CDx.[1]

Working with a partner that offers not only biospecimens but also specialty lab services to test those specimens using different technologies—whether immunochemistry (IHC), polymerase chain reaction (PCR), NGS, or others—for orthogonal characterization can streamline the development process. In addition to meeting regulatory requirements, orthogonal testing is also crucial for eventual reimbursement as payers may look favorably upon such rigorous validation data.

❷ Preclinical studies

In preclinical development, it is common to use histology and IHC to interrogate biospecimens to gain insight into disease biology and pathophysiology.

Case study: Alzheimer disease

Precision for Medicine developed a tissue microarray (TMA) to support preclinical research in Alzheimer disease (AD), the most common cause of dementia. The scientific objective of the TMA was to investigate target and biomarker expression across the continuum of AD. Precision worked collaboratively with our European brain bank partner to source temporal cortex tissue from patients with early, mid-, and late-stage AD and select samples based on additional factors such as amyloid score, apolipoprotein E (APOE) genotype data, cerebrospinal fluid (CSF) pH, and clinical history. We then created a TMA comprising tissues from 5 individual donors per stage and 5 nondisease donors. The resulting TMA was qualified using IHC for expression of hallmark disease markers: phosphorylated tau (phospho-tau, or p-tau) (see Figure 1) and amyloid beta (Aβ) 1-42, a hyperphosphorylated form of Aβ, both of which are found to correlate with AD progression.

The TMA was further characterized by investigating 2 glial markers: IBA-1 for microglia and GFAP for astrocytes, both of which underwent changes in frequency and morphology in different stages of AD. Finally, digital pathology was applied to allow simultaneous evaluation of staining patterns in matched fields of view, enabling detection and quantification of potential targets and biomarkers and their association with specific cell populations (see Figure 2).

Figure 1. **Qualification of an AD TMA using IHC for expression of p-tau**

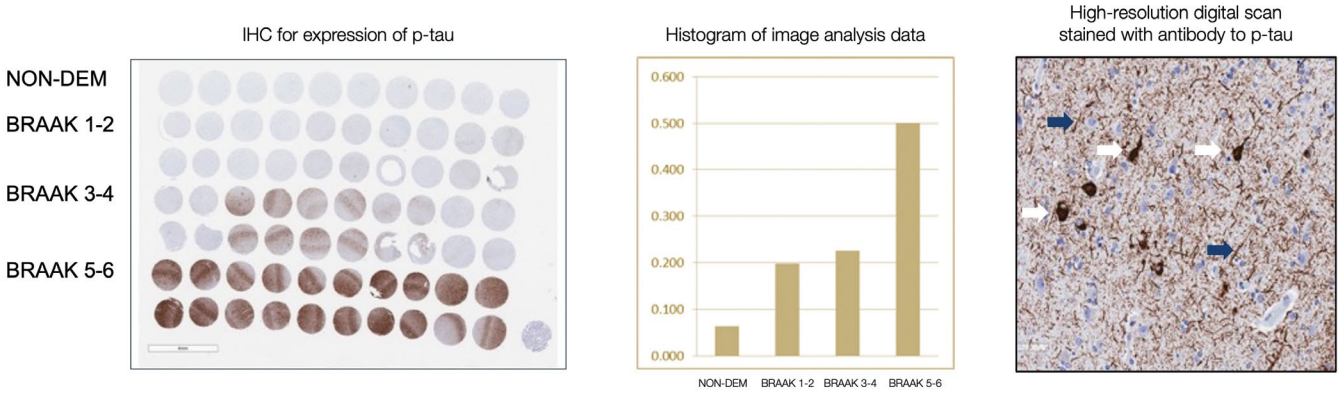
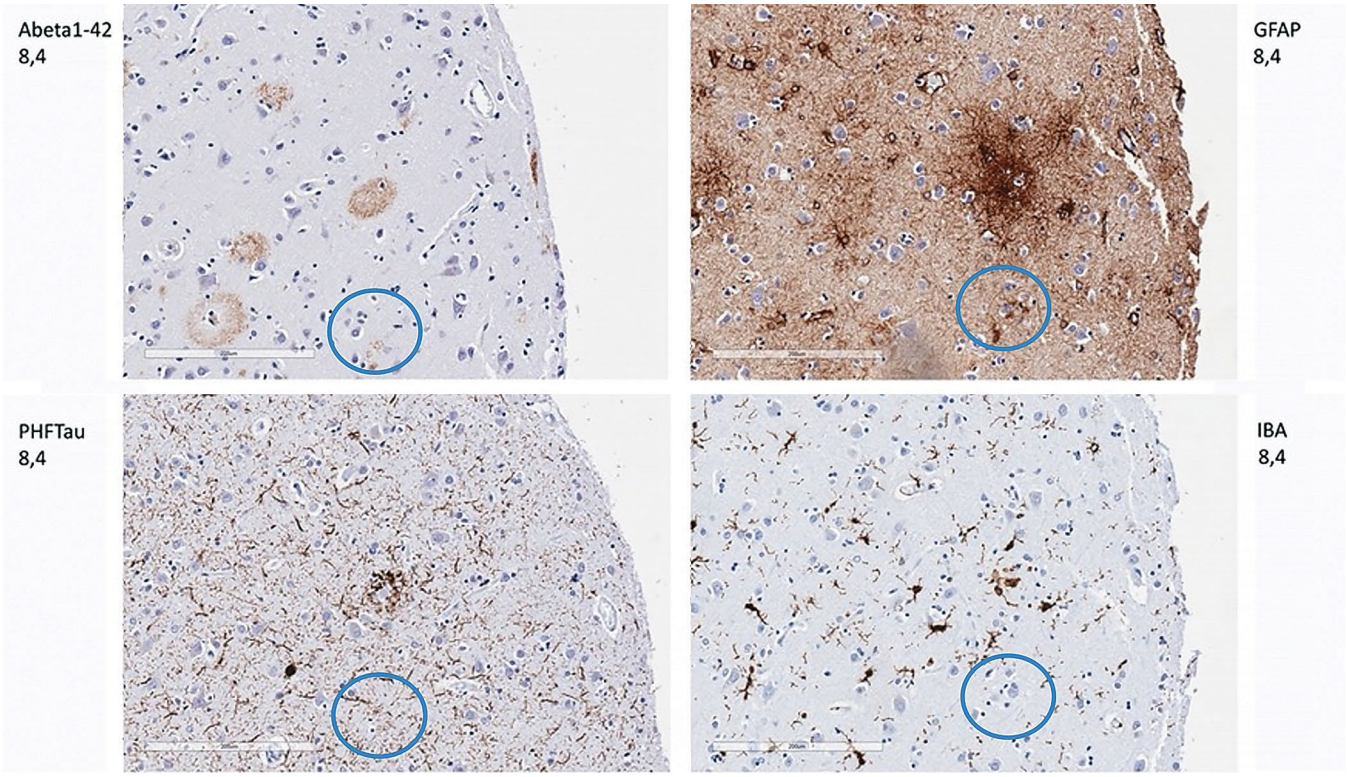


Figure 2. **Evaluation of staining patterns in matched fields of view shows overlapping distribution of hallmark disease and glial markers and dense aggregation of GFAP immunoreactive astrocytes in the location of a suspected plaque**



3 Clinical trials

For clinical trial assays (CTAs) or CDx, the journey from bench to bedside requires characterized biospecimens for

- Determining the prevalence of the biomarker of interest in the target patient population
- Assessing whether the same or similar biomarker is present in healthy individuals or in other diseases
- Evaluating geographic, racial, or ethnic differences in biomarker frequency
- Understanding how disease progression affects biomarker frequency
- Validating that the biomarker of interest is relevant to—or correlates with—the clinical question being asked

For example, in central nervous system (CNS) conditions, development of liquid biopsy-based assays that correlate with brain pathology relies on access to the right biospecimens to support proof of concept and assay validation. Whether it is to obtain CLIA validation, support a clinical trial, or submit for CDx approval—the common denominator for successful assay development and qualification is well-characterized, annotated samples. For instance, qualification of a multiplex immunofluorescence (mIF) IHC assay would require biospecimens for

- Validating the antibodies, including sourcing and titrating the antibodies on biological positive, negative, and internegative control tissues
- Checking the specificity with comparative staining to IHC and against published literature using relevant and appropriate controls
- Optimizing the panel in appropriate control tissues and adjusting the staining and unmixing conditions if necessary
- Qualifying the assay with patient samples relevant to the clinical trial cohort and controls to test intra- and interassay variability

Case study: Clinical oncology diagnostics

The use of NGS in clinical oncology has become a critical diagnostic, prognostic, and treatment decision maker and can be critical to the optimal outcome for patients. Applying NGS assays in a clinical setting requires thorough and rigorous validation of the tests to guarantee proper treatment, regulatory alignment, and reimbursement. However, many characterized samples are required to validate the assay for the detection of the several variant types that can be present on the NGS panel and in the tumor genomes. Rare variant types can be difficult to source and are frequently a bottleneck in getting a new assay ready for patients. To this end, Precision has created sets of validation "kits," collections of characterized samples positive or proven WT for different variants, including fusions, exon deletions, copy number amplifications, MSI, TMB, and rare SNVs to be used by clinical laboratories in the validation of their NGS assays. These kits are collections of previously sequenced tumor FFPE curls cut from blocks that have been QC'd by a pathologist for tumor content and necrosis and are ready for extraction and sequencing by the clinical lab in question.

Precision's library of deeply characterized specimens

In oncology, the development of characterized tumor sample biorepositories is critically important for enabling the development and for streamlining the analytical validation of next-generation sequencing (NGS) and other molecular assays. Critical bottlenecks in the assay validation process include

1. Finding and accessing samples positive for the biomarker of interest
2. Obtaining orthogonal confirmation for positive and negative samples

Screening samples for the presence of relevant variants has traditionally been a labor-intensive and often expensive endeavor. For researchers, having access to specimens accompanied by an easily searchable database of NGS characterization and associated variants available for purchase largely simplifies assay development. In 2020 Precision for Medicine launched a program to prospectively sequence the over 3 million formalin-fixed, paraffin-embedded (FFPE) tissue blocks stored in our biorepository to identify those with mutations of interest for targeted drug development.

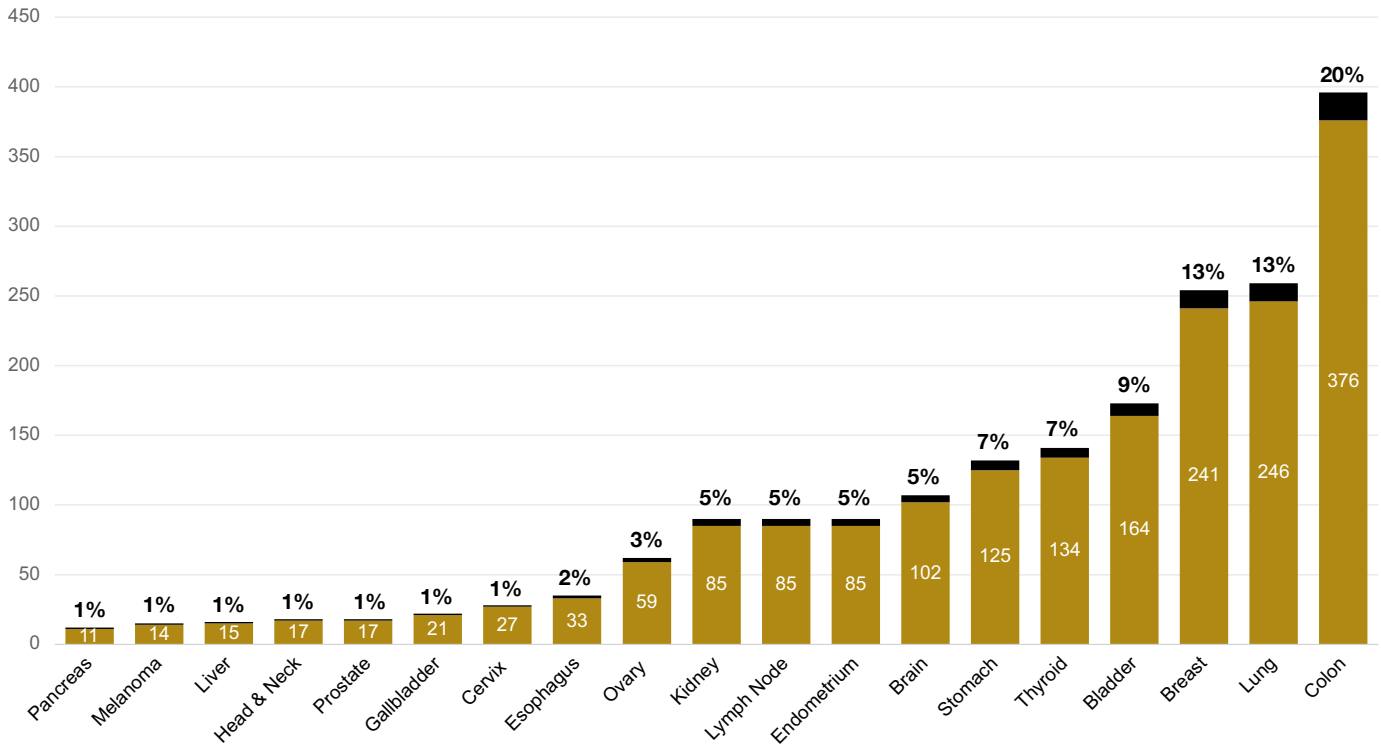
This Precision Oncology Sequencing Initiative (Project P.O.S.I.) is a large-scale, 2-phase initiative. Phase 1 of Project P.O.S.I. focuses on screening FFPE blocks across 12 cancer indications.

- A precision screening project pairing a targeted amplicon-based, 50-gene panel that screens for single nucleotide variants (SNVs), indels, fusions, and amplifications with an integrated NGS platform to enable rapid, accurate sample screening with low

input and limited hands-on time. More than 10,000 FFPE samples have been characterized with this panel across 11 indications, providing novel insights into the distribution of specific variants across cancer types and the co-occurrence or mutual exclusivity of certain variants.

- A pan-cancer screening project using a 500+ gene capture-based panel that screens for tumor mutational burden (TMB), microsatellite instability (MSI), SNVs, indels, fusions, and amplifications for verification, validation, and generation of orthogonal data to support regulatory submissions. As of October 2023, Precision has sequenced over 2000 FFPE samples across 12 cancer indications (see Figure 3).

Figure 3. **Breakdown of sample indications for pan-cancer screening**



Taking it a step further, Precision for Medicine has also scanned hematoxylin and eosin (H&E)–stained slides of every NGS sample. These digital images serve as a supplement to the available physical tissue block, residual extracted DNA and RNA, variant data, and demographic information. They can also be used as training sets for machine learning algorithms that have the potential to detect new targets and biomarkers, thus automating and accelerating discovery.

Phase 2 of Project P.O.S.I. involves ongoing prospective collections for liquid biopsy assays. Approximately 350 donors are enrolled under an investigational review board (IRB)–approved protocol across a range of cancer indications. These donors are screened with both tissue-based and plasma-based assays, creating a biorepository and database of matched tissue-plasma samples. In addition, the donors are re-enrolled at multiple time points, allowing for analysis in changes to their genetic profile over time. Project P.O.S.I. is supported by Precision's robust and expanding genomics and molecular lab infrastructure (see Figure 4). This screening infrastructure was purposefully designed to be platform and assay agnostic, allowing the data to stand alone and enabling objective head-to-head comparisons.

Figure 4. **Precision for Medicine sequencing capabilities**

Equipment	• Illumina NextSeq 550 Dx • Illumina NovaSeq 6000 • Thermo Fisher Genexus • Thermo Fisher QuantStudio 12K Flex • Bio-Rad QX200™ ddPCR™	
Assays	• Thermo Oncomine Precision • Thermo Oncomine Comprehensive • Pillar® oncoReveal™ Multi-Cancer • Illumina TruSight Oncology 500 HT	

Precision's comprehensive support from biospecimen to CDx

A crucial need in the industry is the supply of diverse, highest-quality, IRB-approved, clinically annotated, ready-to-ship human biospecimens, from blood, biofluids, and derivatives to tissues, viable cells, and custom collections (see Figure 5). Among the solutions available, Precision for Medicine is unique among biospecimen providers in obtaining consent for every prospective sample collection. Informing participants on what we are doing, why we are doing it, and how it can benefit other people and science at large is fundamental to our philosophy of ethical sourcing.

Figure 5. **Precision for Medicine biospecimen solutions**

Blood, Biofluids, and Derivatives

Diseased and healthy human blood, plasma, serum, CSF, stool, ascites fluid, saliva, urine, and more.

Tissues

Pathologist-verified fresh, frozen, and fixed tissue specimens from healthy and diseased human subjects.

Viable Cells

HLA-typed cellular products, including PBMCs, BMMCs, Leukopaks, DTCs, and more.

Remnant Diagnostic Specimens

Remnant laboratory specimens characterized by FDA-cleared assays.

Liquid Biopsy

Comprehensive services including kitting, collection, processing, and profiling from your patients or ours.

Custom Biospecimen Collections

Global clinical network, regulatory approved, and ready to enroll.

Our biorepository contains over 10 million biospecimens, and we have 7 laboratories across North America and Europe. Precision also has robust custom collection capabilities, with a clinical network spanning more than 150 investigator sites, 90 therapeutic areas, 25 countries, and 500 actively enrolling studies (see Figure 6).

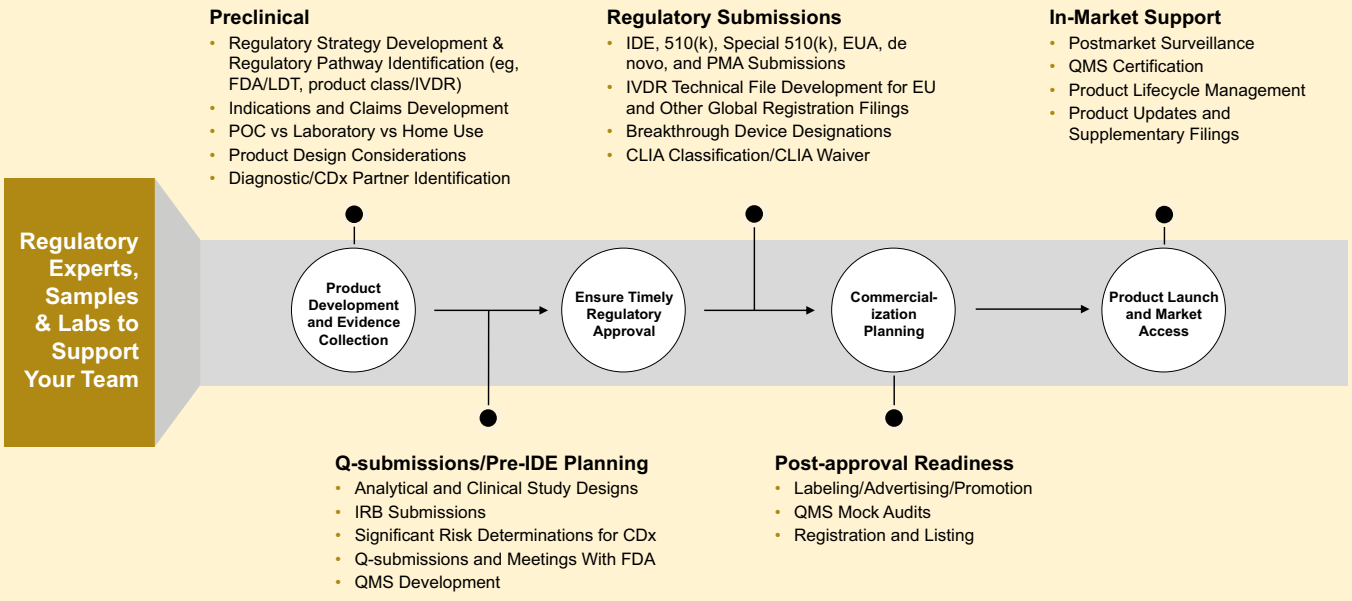
We also offer custom assays to support biomarker development and validation, study kits, and state-of-the-art biobanking facilities for secure storage, management, and distribution of future-use biospecimen assets.

Figure 6. **Precision for Medicine biospecimen capabilities**

150+	90+	25+	60+	500+
INVESTIGATOR SITES	MEDICAL CONDITIONS	COUNTRIES	PHENOTYPIC DATA POINTS	ACTIVELY ENROLLING STUDIES

Partnerships with CDx developers have been instrumental to Precision's success in creating a library of deeply phenotyped, data-rich samples that researchers can leverage to support preclinical and clinical development. CDx developers turn to Precision for high-quality biospecimens, specialty lab capabilities, and the data analysis expertise necessary to extract meaningful insights. Our end-to-end solution for CDx development encompasses regulatory guidance (see Figure 7), orthogonal testing to validate interlaboratory reproducibility, kit assembly, and prospective collections for matched tissue types, minimal residual disease (MRD), and clinical trial enrollment.

Figure 7. **Accelerating assay commercialization from bench to bedside**



Conclusion

Successful drug development hinges upon the selection and availability of high-quality, well-characterized biospecimens. Researchers may benefit from partnering with a biospecimen solutions provider that has the experience and infrastructure to rapidly build extensively characterized, fit-for-purpose specimen sets that have been consented for a broad range of applications.

A true value-add would be a provider that understands not only specimen collection and management but also the scientific rationale behind selecting the right samples at the right time in the research and development cycle.

Reference

1. Abstracts. *J Mol Diagn*. 2022;24(10):S1-S154.

Solving the most complex challenges in biomarker-driven and precision therapeutic and diagnostic development.

Precision for Medicine is the first clinical research services organization engineered to support life sciences companies in the use of biomarkers essential to targeting patient treatments more precisely and effectively. Combining deep scientific expertise, clinical trial excellence, and advanced approaches for data science, Precision accelerates therapeutic development from the late preclinical phase through commercialization.

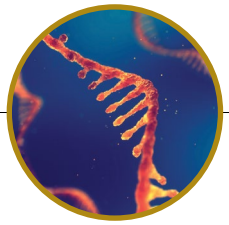
- 7 specialty labs throughout North America and Europe
- Sample processing labs on 5 continents
- Central lab services, including custom kitting, logistics, processing, and storage
- Assays available under GxP, CLIA, CLSI, CAP, ISO 9001/13485

Comprehensive suite of technologies, capabilities, and proprietary approaches to interrogate any sample type

DNA

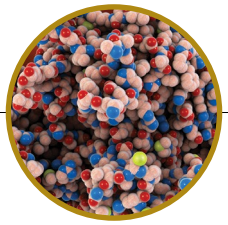
- PCR—ddPCR, qPCR
- NGS—whole exome and targeted resequencing

RNA



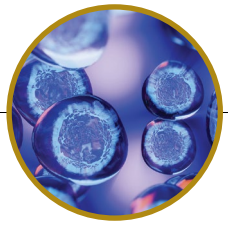
- Gene expression profiling—NanoString
- CAR T and virus (ie, gene therapy) biodistribution
- rtPCR
- MicroRNA analysis

Protein



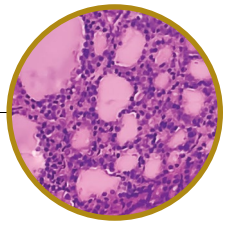
- Comprehensive large molecule bioanalysis—PK, ADA, NAb
- Multiplex cytokine profiling, receptor occupancy, tetramer staining
- Custom ligand binding assays—ELISA, MSD, Biacore
- Quantitative image analysis of protein expression (eg, phosphorylation, signaling)

Cell



- Flow cytometry—up to 31 color panels, ICS, phosphoflow, receptor occupancy
- Functional assays—eg, T cell activation, ADCC, ELISpot
- Single-cell quantitative image analysis
- Proprietary cell separation technology for CTCs and cfDNA
- Immunophenotyping via proprietary epigenetic platform

Tissue



- Multiplex IHC with centralized pathology reading
- Quantitative IF—up to 9 concurrent markers
- FISH, ISH, sequencing

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