ResearchDX

Your Companion in Diagnostics®

JANUARY 2021

ResearchDX

Your Companion in Diagnostics®

Who are we?

- A **CDO** or **Contract Diagnostics Organization**
- HQ: Irvine California
- Founded: 2008
- Founders: Philip D. Cotter, Mathew W. Moore

CDO

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Experienced Leadership

Mathew Moore PhD, Principal

- PhD, biotechnology, UNSW Sydney Australia
- Expertise: Technology assessment, molecular & non-molecular assay development, implementation, commercialization
- Experience: Clinical laboratory (15+ years), research, development, clinical trials, regulatory submissions and approvals

Philip Cotter PhD, FACMG, FFSc(RCPA), Principal

- PhD, biomedical sciences, Mt. Sinai NY
- Expertise: ABMG board-certified; licensed laboratory director (CA, FL, NJ, NY, TN); cytogenetics and molecular genetics
- Experience: Clinical laboratory, regulatory affairs (CLIA, CA, ISO, NY); >100 publications in human genetics

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What is a CDO?

It's like a CRO except, in Diagnostics

A unique animal. There is only one.

- **One partner**. **Complete Biomarker and IVD development resource**.
- We integrate and synchronize all development activities

Experts in all Biomarker and IVD service areas

Drug / IVD alignment of priorities and activities

Speed to regulatory approval / market entry

FDA Submission /Approvals CLIA Validation

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How can a CDO help you?

Companion Dx Development

By solving logistical complexities associated with multi-partner outsourcing

- **Simplifying** — one call when timelines or priorities change
- **Dedicating** — skilled project manager to guide you through all phases of your project

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How can a CDO help you?

Companion Dx Development

By solving logistical complexities associated with multi-partner outsourcing

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IVD Development

By solving gaps in resources and expertise to augment your needs

- **Simplifying** — one call when timelines or priorities change
- **Dedicating** — skilled project manager to guide you through all phases of your project

- **Offering**— Comprehensive menu of services
- **Integrating** —globally accredited / certified clinical services laboratory

PACIFIC DIAGNOSTICS
Seamless Results.

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How are we structured?

Seamless integration and coordination of service groups

Biomarker and Companion Dx development (IVD)

DEVELOPMENT PROCESS OVERVIEW

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Biomarker and Companion Dx development (IVD)

Identify & Finalize Biomarker(s)

- DNA / RNA
- Protein
- Metabolite targets

Develop & Refine IVD Assay

- Screening assay
- Predictive marker
- Final marker selection
- Assay dev, validation & platform selection/ Automation
- CLIA Validation

Validate In Clinical Trials

- Multicenter, prospective trials
- Trials mgmt. & support
- Analysis and meta-analyses
- Reference trial testing services

FDA Regulatory Filing/Approval

- Strategy
- File appropriate regulatory documents per timeline
- FDA interactions

GMP Manufacture Final IVD Kit

- Kitting and manufacture for final clinical product

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Co-development activities

Unlike multi-resourcing, **we are flexible** and coordinate drug and IVD development activities.

When your timelines or priorities shift —as a single resource, **we shift with you**.

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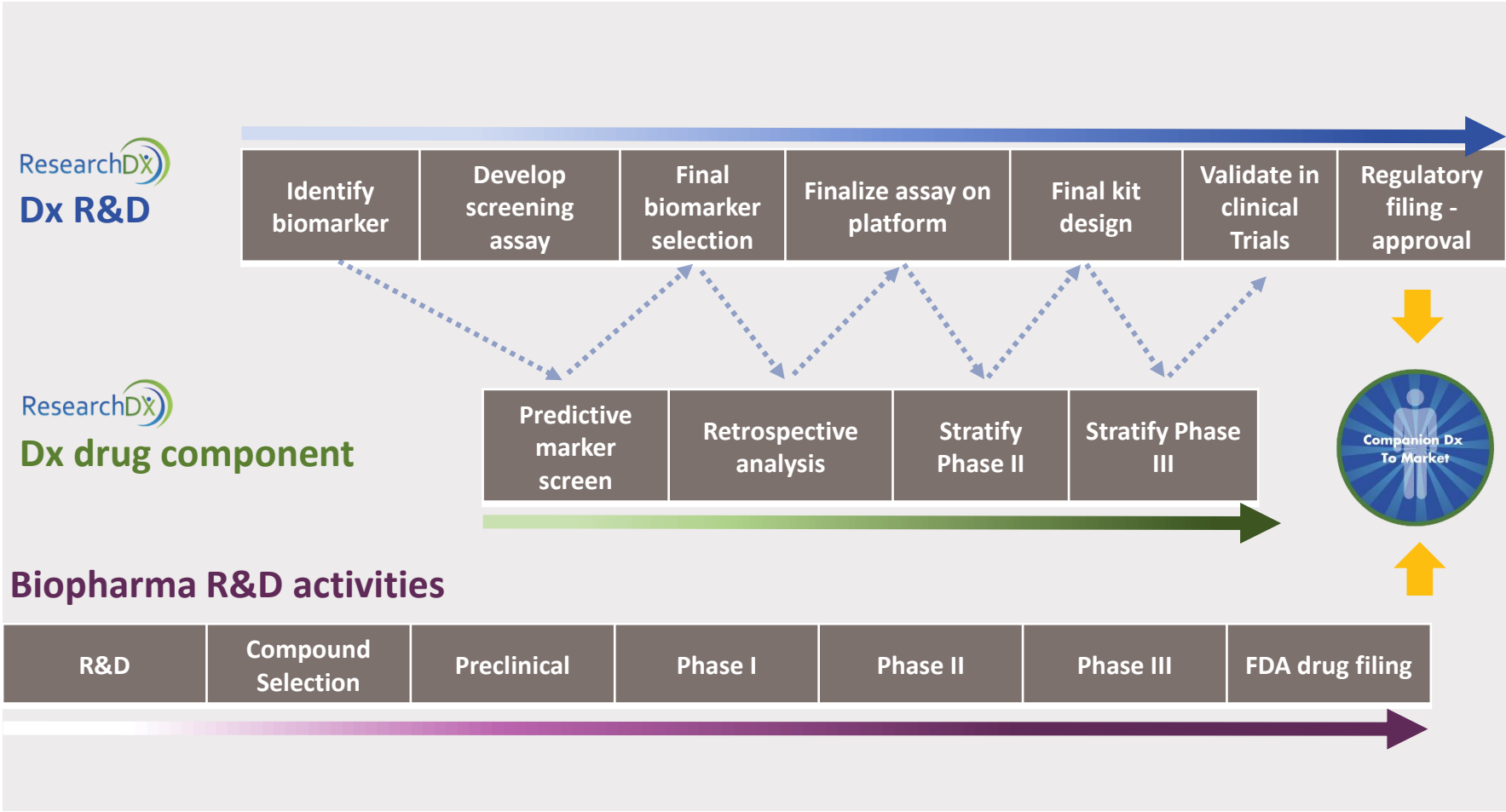
Integrated co-development pathways



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Biomarker and Companion Dx development (IVD)

Flexible, use ResearchDx for any or all services on the developmental roadmap

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Biomarker and IVD development services

Offering complete diagnostic development support, tailored to **your needs**.

Service Divisions

- Research & Development
- Clinical Research
- Regulatory & Compliance
- GMP Contract Manufacturing

Frequently utilized services – all capabilities are not listed.

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Biomarker and IVD development services

Offering complete diagnostic development support, tailored to **your needs**.

Service Divisions

- **Research & Development**
  - Biomarker discovery (molecular, protein, other)
  - Assay / kit development and validation
  - Platform evaluation and automation
  - Studies to establish biological baseline
  - Broad, multi-platform technology capability (without bias)
  - LDT / custom development / clinical validation

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Experienced Leadership

Suman Verma, BAMS, PhD
VP, Genomic Services

- PhD, Molecular biology and biochemistry, University of California, Irvine, CA
- Expertise: Development and validation of molecular assays for clinical implementation (CAP/CLIA) and IVD development
- Experience: Next Gen Sequencing, qPCR, Sanger, Flow Cytometry, Genotyping, histology and control material development

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Capability: genomics technologies

Next Generation Sequencing

- Custom NGS assay development & validation (LDT, CTA, CDx)
- Commercial NGS assay validation (LDT)
- CLIA validated liquid biopsy assays (Roche Avenio ctDNA Expanded, Targeted and Surveillance; Illumina TruSight 500 ctDNA)
- Bioinformatics pipeline development & validation
- Transcriptome sequencing
- Whole exome sequencing
- Whole genome sequencing

Platforms

- Illumina Miseq, NextSeq 500, NovaSeq 600
- Illumina MiSeqDx (IVD)
- Ion Torrent PGM

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Capability: genomics technologies

Quantitative Real-Time PCR (qRTPCR)

- Custom gene expression assay development & validation (LDT, CTA, CDx)
- Custom snp genotyping assay development & validation (LDx, CTA, CDx)
- droplet digital PCR
- Commercial qPCR assay validation (LDT) & testing services
- CLIA validated qPCR assays (MET Exon 14 skipping, BCR/Abl fusion)

Platforms

- Qiagen Rotor-Gene Q MDx (IVD)
- Applied Biosystems® 7900HT
- Applied Biosystems® QuantStudioDx (IVD)
- Thermo fisher Quantstudio 3D Digital PCR system

Capability: genomics technologies

Microarray Technology

- Agilent Microarray scanner (G2600D)
- Affymetrix GeneChip®
- CNV, SNP, Methylation, Expression

FISH: RNA or DNA

- All commercially available probes
- Custom probe design and manufacture
- RUO, IUO and GMP manufactured

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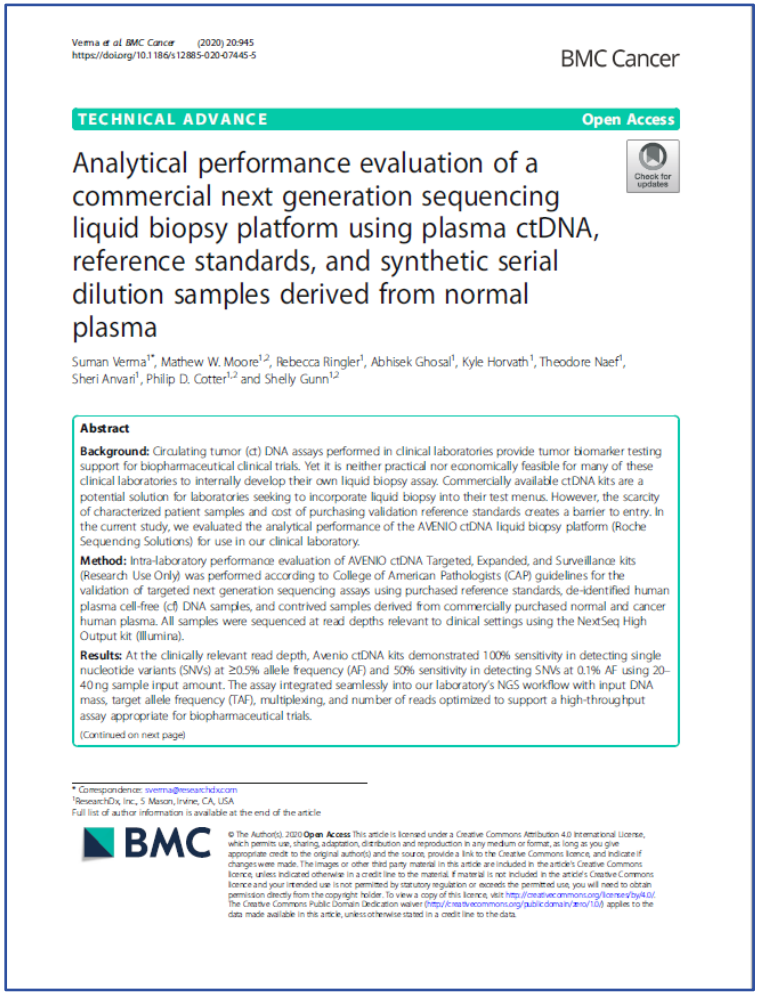
Recent publications in liquid biopsy…



Verma et al. BMC Cancer (2020) 20:945
https://doi.org/10.1186/s12885-020-07445-5

BMC Cancer

TECHNICAL ADVANCE — Open Access

Analytical performance evaluation of a commercial next generation sequencing liquid biopsy platform using plasma ctDNA, reference standards, and synthetic serial dilution samples derived from normal plasma

Suman Verma[1*], Mathew W. Moore[1,2], Rebecca Ringler[1], Abhisek Ghosal[1], Kyle Horvath[1], Theodore Naef[1], Sheri Anvari[1], Philip D. Cotter[1,2] and Shelly Gunn[1,2]

Abstract

Background: Circulating tumor (ct) DNA assays performed in clinical laboratories provide tumor biomarker testing support for biopharmaceutical clinical trials. Yet it is neither practical nor economically feasible for many of these clinical laboratories to internally develop their own liquid biopsy assay. Commercially available ctDNA kits are a potential solution for laboratories seeking to incorporate liquid biopsy into their test menus. However, the scarcity of characterized patient samples and cost of purchasing validation reference standards creates a barrier to entry. In the current study, we evaluated the analytical performance of the AVENIO ctDNA liquid biopsy platform (Roche Sequencing Solutions) for use in our clinical laboratory.

Method: Intra-laboratory performance evaluation of AVENIO ctDNA Targeted, Expanded, and Surveillance kits (Research Use Only) was performed according to College of American Pathologists (CAP) guidelines for the validation of targeted next generation sequencing assays using purchased reference standards, de-identified human plasma cell-free (cf) DNA samples, and contrived samples derived from commercially purchased normal and cancer human plasma. All samples were sequenced at read depths relevant to clinical settings using the NextSeq High Output kit (Illumina).

Results: At the clinically relevant read depth, Avenio ctDNA kits demonstrated 100% sensitivity in detecting single nucleotide variants (SNVs) at ≥0.5% allele frequency (AF) and 50% sensitivity in detecting SNVs at 0.1% AF using 20–40 ng sample input amount. The assay integrated seamlessly into our laboratory's NGS workflow with input DNA mass, target allele frequency (TAF), multiplexing, and number of reads optimized to support a high-throughput assay appropriate for biopharmaceutical trials.

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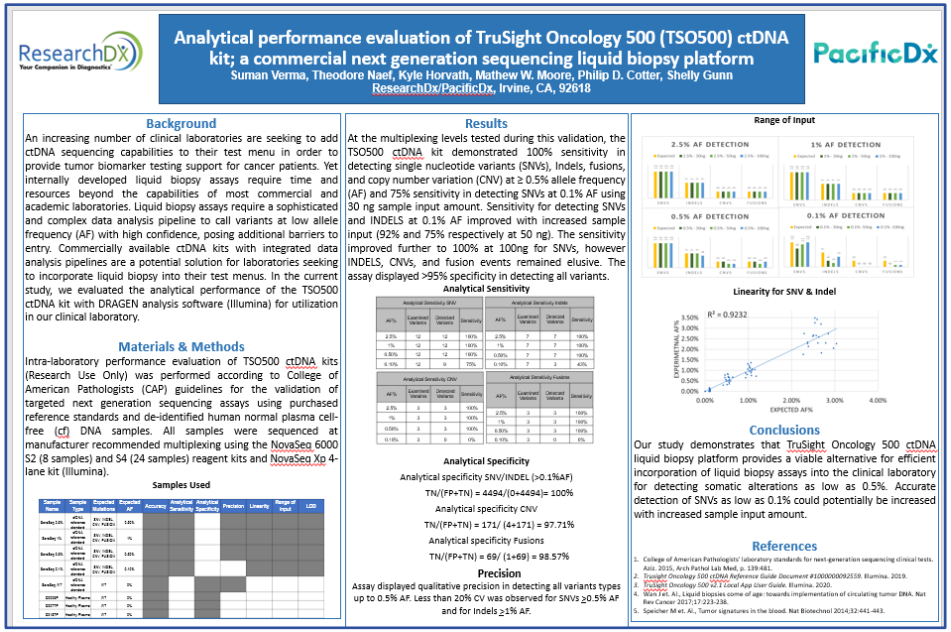

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Analytical performance evaluation of TruSight Oncology 500 (TSO500) ctDNA kit; a commercial next generation sequencing liquid biopsy platform

Suman Verma, Theodore Naef, Kyle Horvath, Mathew W. Moore, Philip D. Cotter, Shelly Gunn
ResearchDx/PacificDx, Irvine, CA, 92618

PacificDx

Background

An increasing number of clinical laboratories are seeking to add ctDNA sequencing capabilities to their test menu in order to provide tumor biomarker testing support for cancer patients. Yet internally developed liquid biopsy assays require time and resources beyond the capabilities of most commercial and academic laboratories. Liquid biopsy assays require a sophisticated and complex data analysis pipeline to call variants at low allele frequency (AF) with high confidence, posing additional barriers to entry. Commercially available ctDNA kits with integrated data analysis pipelines are a potential solution for laboratories seeking to incorporate liquid biopsy into their test menus. In the current study, we evaluated the analytical performance of the TSO500 ctDNA kit with DRAGEN analysis software (Illumina) for utilization in our clinical laboratory.

Materials & Methods

Intra-laboratory performance evaluation of TSO500 ctDNA kits (Research Use Only) was performed according to College of American Pathologists (CAP) guidelines for the validation of targeted next generation sequencing assays using purchased reference standards and de-identified human normal plasma cell-free (cf) DNA samples. All samples were sequenced at manufacturer recommended multiplexing using the NovaSeq 6000 S2 (8 samples) and S4 (24 samples) reagent kits and NovaSeq Xp 4-lane kit (Illumina).

Samples Used

Results

At the multiplexing levels tested during this validation, the TSO500 ctDNA kit demonstrated 100% sensitivity in detecting single nucleotide variants (SNVs), Indels, fusions, and copy number variation (CNV) at ≥ 0.5% allele frequency (AF) and 75% sensitivity in detecting SNVs at 0.1% AF using 30 ng sample input amount. Sensitivity for detecting SNVs and INDELS at 0.1% AF improved with increased sample input (92% and 75% respectively at 50 ng). The sensitivity improved further to 100% at 100ng for SNVs, however INDELS, CNVs, and fusion events remained elusive. The assay displayed >95% specificity in detecting all variants.

Analytical Sensitivity

Analytical Specificity

Analytical specificity SNV/INDEL (>0.1%AF)

TN/(FP+TN) = 4494/(0+4494)= 100%

Analytical specificity CNV

TN/(FP+TN) = 171/ (4+171) = 97.71%

Analytical specificity Fusions

TN/(FP+TN) = 69/ (1+69) = 98.57%

Precision

Assay displayed qualitative precision in detecting all variants types up to 0.5% AF. Less than 20% CV was observed for SNVs ≥0.5% AF and for Indels ≥1% AF.

Range of Input

2.5% AF DETECTION | 1% AF DETECTION | 0.5% AF DETECTION | 0.1% AF DETECTION

Linearity for SNV & Indel

$R^2 = 0.9232$

EXPERIMENTAL AF% / EXPECTED AF%

Conclusions

Our study demonstrates that TruSight Oncology 500 ctDNA liquid biopsy platform provides a viable alternative for efficient incorporation of liquid biopsy assays into the clinical laboratory for detecting somatic alterations as low as 0.5%. Accurate detection of SNVs as low as 0.1% could potentially be increased with increased sample input amount.

References

1. College of American Pathologists' laboratory standards for next-generation sequencing clinical tests. Arch Pathol Lab Med, p. 139-481.
2. *TruSight Oncology 500 ctDNA Reference Guide Document #1000000092159*. Illumina. 2019.
3. *TruSight Oncology 500 v2.1 Local App User Guide*. Illumina. 2020.
4. Wan J.et. Al., Liquid biopsies come of age: towards implementation of circulating tumor DNA. Nat Rev Cancer 2017;17:223-238.
5. Speicher M et. Al., Tumor signatures in the blood. Nat Biotechnol 2014;32:441-443.

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Capability: Protein analysis and cell culture

- Cell culture facility (100+ characterized control cell lines, BSLII facility, incubators, liquid nitrogen storage)
- Contrived cell line controls (FFPE Cell blocks/ nucleic acid mix/ cell pellets)
- Flow Cytometry (Commercial and custom flow panel for hematology and lymphoid malignancies)
- Anatomic Pathology (IHC, H&E on FFPE and Frozen tissue, Pathology review and macro-dissection)
- Cytogenetics

Platforms

- Beckman Coulter FC500 (IVD)
- Metasystems Metafer imaging station
- Carl Zeiss Auto Imager

PACIFIC DIAGNOSTICS Seamless Results.

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Capability: Protein Analysis

Diverse technologies support IVD development and scale-up activities that may require automated DNA extraction and liquid-handling systems

ELISA (Immunoassay)

- Direct and Sandwich ELISA
- Identify and qualify commercial fit for purpose ELISA kits for PK & PD studies
- Wide applicability for assay development
- Analyte detection e.g cytokines

Microplate Readers

- Tecan GENios Pro®
- Dynex® MRX

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Biomarker and IVD development services

Offering complete diagnostic development support, tailored to **your needs**.

Service Divisions

- **Clinical Research**
  - Complete clinical trial services, program management and support
  - Clinical trial strategic consulting
  - Reference trial testing services
  - Clinical primary site testing
  - Analytical and clinical testing
  - Complete data management program support

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Experienced Leadership

Shelly Gunn MD PhD, CMO, Laboratory Director

- MD and PhD, molecular biology, UTHSCSA Medical School, San Antonio TX
- Expertise: Board-certified in clinical pathology; CA, TX, NY medical licensure; NY state certificate of qualification in molecular and cellular tumor markers
- Experience: Medical laboratory director, 15+ years

Lony Lim PhD, SI (ASCP), VP Operations

- BS and PhD, molecular biology, University of Wisconsin-Madison,
- Expertise: CLIA and IVD Development
- Experience: 20 plus years including VP Lab Ops. GenomeDx, PLUS Diagnostics, and CombiMatrix Diagnostics. Also Miraca Life Sciences, US Labs, Ameripath Specialty Labs, and Luminex
- Currently supports GLP, Clinical Testing and Product Manufacturing

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Experienced Leadership

Jayne Scoggin BA, CT(ASCP), CG(ASCP), Director Quality Management

- Cytotechnology University of Oklahoma Health Sciences Center, Oklahoma City, OK
- Expertise: CA Cytogenetics and cytotechnology licensure
- Experience: Clinical laboratory science, quality management / quality assurance, laboratory operations and regulatory affairs (30+ years)

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About our clinical laboratory services

ResearchDx maintains licensure, accreditation, certification and compliance where applicable to pertinent regulatory bodies and to local, state and federal laws.

- CLIA-certified
- College of American Pathologists (CAP) accredited
- California licensure
- High level of knowledge, experience and expertise

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IVD development services

Offering complete diagnostic development support, tailored to **your needs**.

Service Divisions

- **Regulatory & Compliance**
  - Strategy and FDA interactions
  - IVD PRE-IDE, PMA and all types of 510(k) filings
  - International regulatory filings
  - Compliance auditing and consulting
  - Compliant labeling
  - Software documentation
  - Third party review by Accredited Persons (AP)

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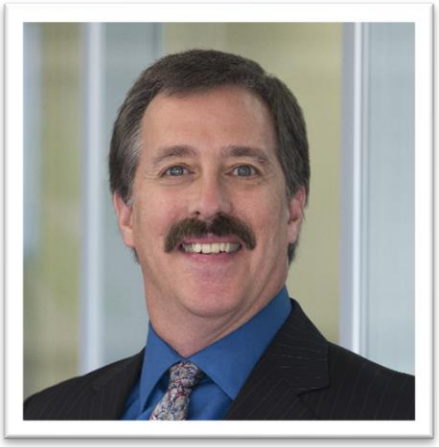
Experienced Leadership



Louis Ferland PhD, PMP, VP Clinical Research Services

- PhD, molecular biology, PMP certification (PMI)
- Expertise: Regulatory, FDA submissions, GLP/GMP/GCP, project management, assay development and validation
- Experience: Project management, clinical research, regulatory affairs, research and development (30+ years)

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IVD development services

Offering complete diagnostic development support, tailored to **your needs**.

Service Divisions

- **GMP Contract Manufacturing**
  - Custom reagents, assays or final kits for research, clinical, final product
  - Molecular / non-molecular IVD development
  - OEM reagents, kit components or assays
  - Certificates of Analysis (COA)
  - Sample collection kits

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Our reputation is built on success.

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Thank You

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