SPECIMEN REQUIREMENTS

	Bone Marrow	Leukemic Blood	Isolated Genomic DNA	FFPE Tissue	Tissue or fluid in PreservCyt
Hematologic Malignancies Panel	X	X	X		X
Solid Tumor Panel			X	X	X
Penn Precision Panel			X	X	X
Fusion Transcript Panel				X	X
Lymphoma Panel	X	X	X	X	X

Given the analytical sensitivity of the assay, specimens must contain a minimum of 10% tumor nuclei across the entire tissue. Submitted specimens must contain a copy of the corresponding pathology report.

CONTACT

Kojo S. Elenitoba-Johnson, MD
Director
Center for Personalized Diagnostics
E: Kojo.Elenitoba-Johnson@uphs.upenn.edu

Jennifer Morrissette, PhD
Clinical Director
Center for Personalized Diagnostics
T: 215.615.2387
E: Jennifer.Morrissette@uphs.upenn.edu

David B. Lieberman, MS, CGC
Technical Manager
Center for Personalized Diagnostics
T: 215.349.8416
E: David.Lieberman@uphs.upenn.edu

For more information please contact
The Center for Personalized Diagnostics
215.615.3966
PennMedicine.org/CPD

SPECIMEN TYPES

Bone Marrow

Requirements: 2-4 cc drawn in an EDTA (purple-top) tube.

Transport Conditions: Transport at ambient temperature (18-25°C / 64-77°F) in an insulated container. Specimens should arrive in the laboratory within 48 hours of collection. Do not freeze.

Formalin Fixed, Paraffin Embedded Tissue (FFPE Tissue)

Requirements: Less than 50% tumor nuclei in sample: 10-15 unstained 5 µM FFPE slides containing adequate amounts of tumor to be analyzed. Areas containing tumor must be marked on an adjacent H & E slide (outside cases). Greater than 50% tumor nuclei in sample: 6 to 9 rolls cut at 10 µM and placed in a 1.5 ml tube. All samples must come with a corresponding H&E slide from the top and bottom of the sample. All samples must include a copy of the surgical pathology report. Specimens fixed or processed with alternative fixatives will result in DNA that fails QC and therefore will be rejected. Specimens containing less than 15% total tumor nuclei will also be rejected.

Transport Conditions: Transport at ambient temperature (18-25°C / 64-77°F) in an insulated container by overnight courier. Do not heat or freeze. Avoid direct exposure to light.

Leukemic Blood

Requirements: 3-5 cc drawn in an EDTA (purple-top) tube. (White blood cell count > 10,000 cells/mL with at least 15% circulating blasts or malignant cells.)

Transport Conditions: Transport at ambient temperature (18-25°C / 64-77°F) in an insulated container. Specimens should arrive in the laboratory within 48 hours of collection. Do not freeze.

Isolated Genomic DNA

Requirements: 20 µL at a minimum of 35 ng/µL determined by a fluorescent based assay (i.e. Qubit, picogreen). All DNA received by the laboratory not meeting our quality control standards will not be tested and an inadequate specimen report will be generated. Must be isolated in a certified CLIA laboratory.

Transport Conditions: Transport at ambient temperature (18-25°C / 64–77°F) in an insulated container by overnight courier. Specimen should arrive in the laboratory within 48 hrs of collection.

Fine Needle Aspirate Rinse Material containing Malignancy (confirmed with on-site evaluation by Penn Medicine cytopathology or final interpretation)

Requirements: Greater than 10% tumor nuclei in sample (on smears or liquid-based cytology slide or cell block slides). PreservCyt vial prepared for potential molecular testing from Cytopathology sent directly to CPD within three weeks of original collection date. (Note, FNA cell blocks if adequate can be utilized longer than 3 weeks).

Transport Conditions: Transport at ambient temperature (18-25°C/64-77°F). Do not freeze. Specimens can only be used within three weeks of original collection date.

Malignant Effusions, Liquid

Requirements: Greater than 10% tumor nuclei in sample confirmed by a Penn Medicine cytopathology evaluation (on liquid based cytology slide or cell block slides). PreservCyt vial prepared for potential molecular testing from Cytopathology sent directly to CPD within three weeks of original collection date. (Note, a malignant effusion cell block if adequate can be utilized longer than 3 weeks; follow formalin fixed, paraffin embedded tissue specimen type).

Transport Conditions: Transport at ambient temperature (18-25°C/64-77°F). Do not freeze. Specimens can only be used within three weeks of original collection date.

For more information please contact The Center for Personalized Diagnostics at **215.615.3966** or visit **PennMedicine.org/CPD**

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The Center for PERSONALIZED DIAGNOSTICS

Precision Diagnostics for Personalized Medicine

PennMedicine.org/CPD | 215.615.3966

The Center for Personalized Diagnostics (CPD) is a joint initiative between Penn Medicine's Department of Pathology and Laboratory Medicine and the Abramson Cancer Center. The Center integrates molecular genetics, pathology informatics and genomic pathology to develop personalized diagnostic profiles for individuals with cancer. The CPD offers the highest volume of genome testing in the region.

PENN MEDICINE CPD SERVICES

Using customized computational methods, including large-scale, massively parallel DNA sequencing and chromosomal analysis, the CPD identifies personal mutation signatures for distinct tumor subtypes.

Penn's Center for Personalized Diagnostics is a CAP/CLIA certified laboratory and offers the following precise cancer gene-sequencing panels:

- **Hematologic malignancy panel,** containing 68 genes and focused primarily on focused primarily on AML, MDS and CLL
- **Comprehensive solid tumor panel,** containing 153 genes known to be mutated in a wide range of tumor types
- **Penn Precision Panel,** with a subset of 20 genes if sample is not adequate for full panel
- **FusionTranscript Panel 2.0** containing 56 genes
- **PennLYMPHOMA** containing 40 genes

HEMATOLOGIC MALIGNANCIES PANEL

ABL1	*CEBPA***	*GATA2*	*MAP2K1*	*NPM1*	*RUNX1*	*TP53*
ASXL1	*CSF1R*	*GNAS*	*MAPK1*	*NRAS*	*SETBP1*	*TPMT*
ATM	*CSF3R*	*HNRNPK*	*MIR142*	*PDGFRA*	*SF1*	*U2AF1*
BCOR	*DDX3X*	*IDH1*	*MPL*	*PHF6*	*SF3A1*	*U2AF2*
BCORL1	*DNMT3A*	*IDH2*	*MYC*	*POT1*	*SF3B1*	*WT1*
BIRC3	*ETV6*	*IL7R*	*MYCN*	*PRPF40B*	*SMC1A*	*XPO1*
BRAF	*EZH2*	*JAK2*	*MYD88*	*PTEN*	*SRSF2*	*ZMYM3*
CALR	*FAM5C*	*KIT*	*NF1*	*PTPN11*	*STAG2*	*ZRSR2*
CBL	*FBXW7*	*KLHL6*	*NOTCH1*	*RAD21*	*TBL1XR1*	
CDKN2A	*FLT3*	*KRAS*	*NOTCH2*	*RIT1*	*TET2*	

- **Accepted Specimens:** Blood; bone marrow; fresh tissue in PreservCyt
- **Minimum Requirements:** 10% tumor nuclei for tissue, 100ng of DNA
- **Covers:** Clinically relevant hotspots only
- **Detects:** Single nucleotide variants (SNVs); small indels; copy number gain of select targets
- **Limitations:** No indels > 85bp; no deep intronic splice variants; no promoter variants; no structural rearrangements; no methylation

***CEBPA is analyzed only when a diagnosis of AML is provided*

LYMPHOMA PANEL

ATM	*CD79A*	*EZH2*	*MAP2K1*	*PLCG2*	*SOCS1*	*TNFRSF14*
B2M	*CD79B*	*GNA13*	*MYD88*	*POT1*	*STAT3*	*TP53*
BIRC3	*CIITA*	*ID3*	*NFKBIE*	*RHOA*	*STAT5B*	*TRAF3*
BRAF	*CREBBP*	*IDH2*	*NOTCH1*	*RPS15*	*TCF3*	*XPO1*
BTK	*CXCR4 (CD184)*	*JAK3*	*NOTCH2*	*RRAGC*	*TET2*	
CARD11	*EGR2*	*KLF2*	*PLCG1*	*SF3B1*	*TNFAIP3*	

- **Accepted Specimens:** Blood; bone marrow; formalin-fixed, paraffin-embedded (FFPE) tissue; fresh tissue in PreservCyt
- **Minimum Requirements:** 10% tumor nuclei for tissue, 10ng of DNA
- **Covers:** Clinically relevant hotspots only
- **Detects:** Single nucleotide variants (SNVs); small indels
- **Limitations:** No indels > 25bp; no deep intronic splice variants; no promoter variants; no structural rearrangements; no methylation

FUSION TRANSCRIPT PANEL

AKT1	*CCND1*	*EWSR1*	*JAZF1*	*NTRK1*	*PTH*	*TCF12*
ALK	*CIC*	*FGFR1*	*KRT20*	*NTRK2*	*RAF1*	*TERT*
AXL	*EGFR*	*FGFR2*	*KRT7*	*NTRK3*	*RET*	*TFE3*
BCOR	*EML4*	*FGFR3*	*MEAF6*	*PDGFB*	*ROS1*	*TFG*
BRAF	*EPC1*	*FOXO1*	*MET*	*PIK3CA*	*SLC5A5*	*THADA*
CALCA	*ERBB2*	*FUS*	*MKL2*	*PLAG1*	*SS18*	*TMPRSS2*
CAMTA1	*ERG*	*GLI1*	*NCOA2*	*PMS2*	*STAT6*	*USP6*
CCNB3	*ESR1*	*HMGA2*	*NRG1*	*PPARG*	*TAF15*	*YWHAE*

- **Accepted Specimens:** Formalin-fixed, paraffin-embedded (FFPE) tissue; fresh tissue in PreservCyt
- **Minimum Requirements:** 10% neoplastic tissue
- **Covers:** Selected exon- boundaries
- **Detects:** Aberrant transcripts involving the included exons; can detect novel fusion partners at known break-points
- **Limitations:** Only detects fusions which include at least one of the targets at the included exons; no SNVs; no copy number changes; no small indels; no methylation

PENN PRECISION PANEL

AKT1	*EGFR*	*HRAS*	*KIT*	*MET*	*PDGFRA*	*RET*
ALK	*ERBB2*	*IDH1*	*KRAS*	*NRAS*	*PIK3CA*	*TP53*
BRAF	*CSF1R*	*IDH2*	*MAP2K1*	*NOTCH1*	*PTEN*	

- **Accepted Specimens:** Formalin-fixed, paraffin-embedded (FFPE) tissue; fresh tissue in PreservCyt
- **Minimum Requirements:** 10% neoplastic tissue
- **Covers:** Hotspots and the entire coding sequence of TP53
- **Detects:** Single nucleotide variants (SNVs); small indels; some copy number gains
- **Limitations:** Lower limit of reportability 5% variant allele fraction (VAF); Indels unreliable > 30bp; no deep intronic splice variants; no promoter variants; no structural rearrangements; no methylation

SOLID TUMOR PANEL

ABL1	*CCND3*	*ESR1*	*JAK3*	*MRE11A*	*PIK3CA*	*SMARCA4*
AKT1	*CCNE1*	*ESR2*	*KDM5A*	*MSH2*	*PIK3CB*	*SMO*
AKT2	*CDH1*	*EZH2*	*KDM5C*	*MSH6*	*PIK3R1*	*SPOP*
AKT3	*CDK4*	*FBXW7*	*KDM6A*	*MTOR*	*PTCH1*	*SRC*
ALK	*CDK6*	*FGF3*	*KDR*	*MYC*	*PTEN*	*STAG2*
APC	*CDKN2A*	*FGFR1*	*KIT*	*MYCN*	*PTPN11*	*STK11*
AR	*CHEK2*	*FGFR2*	*KMT2C*	*NBN*	*RAB35*	*SUFU*
ARAF	*CIC*	*FGFR3*	*KRAS*	*NF1*	*RAC1*	*SUZ12*
ARID1A	*CRKL*	*FGFR4*	*MAP2K1*	*NF2*	*RAD50*	*SYK*
ARID2	*CSF1R*	*FLT3*	*MAP2K2*	*NTRK1*	*RAD51*	*TERT*
ATM	*CTNNB1*	*FUBP1*	*MAP2K4*	*NTRK2*	*RAD51B*	*TET2*
ATRX	*DAXX*	*GATA3*	*MAPK1*	*NTRK3*	*RAD51C*	*TGFBR2*
AURKA	*DDR2*	*GNA11*	*MAPK3*	*NKX2-1*	*RAD51D*	*TP53*
BAP1	*DNMT3A*	*GNAQ*	*MAX*	*NOTCH1*	*RAF1*	*TRAF7*
BRAF	*EGFR*	*GNAS*	*MCL1*	*NOTCH2*	*RB1*	*TSC1*
BRCA1	*EIF1Ax*	*HRAS*	*MDM2*	*NOTCH3*	*RET*	*TSC2*
BRCA2	*EPHA3*	*H3F3A*	*MDM4*	*NRAS*	*RHOA*	*TSHR*
BRIP	*ERBB2*	*IDH1*	*MED12*	*EP300*	*RNF43*	*U2AF1*
BTK	*ERBB3*	*IDH2*	*MEN1*	*PAK1*	*SETD2*	*VHL*
CBP	*ERBB4*	*IGF1R*	*MET*	*PALB2*	*SF3B1*	*WT1*
CCND1	*ERCC2*	*JAK1*	*MITF*	*PBRM1*	*SLIT2*	*XRCC2*
CCND2	*ERG*	*JAK2*	*MLH1*	*PDGFRA*	*SMAD4*	

- **Accepted Specimens:** Formalin-fixed, paraffin-embedded (FFPE) tissue; fresh tissue in PreservCyt
- **Minimum Requirements:** 10% neoplastic tissue
- **Covers:** Entire coding sequence +/- ~10bp flanking intronic sequence of listed genes (see above)
- **Detects:** Single nucleotide variants (SNVs); small indels
- **Limitations:** Lower limit of reportability 5% variant allele fraction (VAF); Indels unreliable > 30bp; no deep intronic splice variants; no promoter variants; no structural rearrangements; no methylation

REPORTS

Reports include all variants found in the tested specimen that are not supported by the literature as germline population variants. These variants are classified into one of two categories: 1) disease-associated variants or 2) variants of uncertain significance (VOUS). Benign population variants are not reported.

Report categories for DNA-based tests, include abnormal, variant, normal, and no result based upon the types of variants detected. Report categories for the Fusion Transcript Panel include positive, negative and no result. The evidence of wild-type and variant reads supporting each of the reported variants is included in the interpretation to aid in understanding the relative proportions of different variants seen in the specimen.

RESULTS

Results from these studies and clinical testing demonstrate the utility of using multi-analyte approaches to identify mutations across a wide range of tumor types. Using a targeted next-generation sequencing test looking across multiple known cancer-related genes, many different mutation types can be simultaneously detected. Across each major tumor type, disease-associated mutations impacting diagnosis, prognosis and therapy-related treatment decisions can be found.

CYTOPATHOLOGY

For cytology samples, FNA rinses, body fluids, and cell blocks can be used. FNA rinses can yield better-quality DNA for the full solid tumor panel as well as quicker turnaround times. For any specimen type where a full solid tumor panel cannot be performed, the Penn Precision Panel (PPP) will be attempted as determined by CPD. The PPP will test for 20 commonly mutated genes in many solid tumors even in samples of frequent low or poor quality DNA yield. This extremely targeted panel can be used to reflex samples that have insufficient DNA yield, to run on larger panels with as little as one nanogram of input DNA and can be performed on a range of cytology specimens, including FNA rinse specimens, as well as limited biopsy specimens. PPP results show the same rate of abnormal results as the larger, full panel with high-quality DNA.

"The CPD's tests reveal the genetic blueprint of each patient's tumor. This genetic data empowers clinical oncologists to take an individualized approach to cancer care, giving them the tools to refine diagnosis, provide better prognostication, adjust treatment plans according to the genetic makeup of the cancer, and identify a more appropriate selection of targeted therapies—saving lives and spending health resources more wisely."

– **DAVID B. ROTH, MD, PHD**
Simon Flexner Professor and
Chair of Pathology and Laboratory Medicine
Director, Penn Medicine Precision Medicine Program

DATA FROM 10,000 PATIENTS ANALYZED