CC-SIGN® Hematopathology Next-Generation Sequencing (NGS) Panels

Background

Recurrent somatic mutations and fusions are found in numerous hematologic neoplasms, including acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid neoplasms, myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB* or *FGFR1*, and selected lymphoproliferative disorders.

Identifying such mutations provides pathologists and clinicians with valuable data that may assist in the diagnosis, classification, prognostic evaluation, and therapeutic management of these malignancies.

Mutational data in these disorders has been incorporated into the current diagnostic criteria of the World Health Organization (WHO) Classification of Hematopoietic and Lymphoid Tissues and into practice guidelines from the National Comprehensive Cancer Network (NCCN).

CC-SIGN® NGS panels include:

- **Acute Leukemia**
  Combined detection of DNA (single nucleotide variants, small insertions, and deletions) and RNA (gene fusions)
- **Chronic Myeloid Neoplasms**
  DNA variants only
- **Hematologic Neoplasm Fusion**
  RNA gene fusions only

In addition to the panels listed above, Cleveland Clinic also offers the following smaller subpanels for focused disease testing:

- **Myeloproliferative Neoplasms Panel**
  Detects variants in the three (3) common genes associated with myeloproliferative neoplasms: *JAK2* V617F, *JAK2* exon 12, *MPL* exon 10, and *CALR* exon 9
- **Chronic Lymphoproliferative Disorders Panel**
  A seven (7) gene panel that includes *BRAF, MYD88, NOTCH1, SF3B1, STAT3, STAT5B*, and *TP53* (targeted regions outlined in **Tables 1 & 3**) intended for patients with known or suspected mature lymphoid leukemias, including chronic lymphocytic leukemia, lymphoplasmacytic leukemia, hairy cell leukemia, and large granular lymphocyte leukemias

Details of the regions covered in all panels are listed in the Test Directory on clevelandcliniclabs.com.

Whole genome copy number and loss of heterozygosity analysis may be obtained by ordering **Leukemic Blood Cancer Chromosome Microarray + SNP** (*BLLSNP*) or Bone Marrow Cancer Chromosome Microarray + *SNP (BMHSNP)*.

Acute Leukemia Next-Generation Sequencing Panel

Overview

The Acute Leukemia Next-Generation Sequencing Panel analyzes the clinically relevant regions of 107 genes (RNA, gene fusions and structural variants) and 63 genes (DNA, single nucleotide variants, small insertions, and deletions) known to be mutated or rearranged in hematologic neoplasms. Performed on peripheral blood, bone marrow aspirates, FFPE tissues, and clot sections, this test identifies abnormalities in the targeted genes (refer to **Tables 1 & 2**).

For NGS testing on limited specimens from patients with known or suspected acute leukemia, a partial panel may be performed: please refer to either the **Chronic Myeloid Neoplasms Next-Generation Sequencing Panel** (for AMLs) or **Hematologic Neoplasm Fusion Next-Generation Sequencing Panel** (for ALLs).

Clinical Indications

This assay is intended for molecular evaluation of known or suspected acute leukemia (myeloid, lymphoblastic, ambiguous lineage) to aid in the diagnosis, classification, prognosis, and/or therapeutic options of these entities.

Interpretation

Variants are classified according to established guidelines and an interpretation is provided. Reported variants include those of strong or potential clinical significance and variants of unclear clinical significance. Common population variants are not included in the report.

Methodology & Limitations

Nucleic acid, both DNA and RNA, extracted from the specimen is subjected to anchored Multiplex PCR-based target enrichment and sequenced using Illumina (San Diego, CA) chemistry.

Based on validation, the DNA testing delivered an average of >500X coverage, and >98% of targeted regions showed over 100X coverage. The test demonstrated 95.2% sensitivity and 99.9% specificity in identifying single nucleotide variants, small insertions, and deletions (indels) (less than 10bp) of >5% variant allele fraction (VAF). For the identification of large indels (greater than 10bp) >5% VAF, including *FLT3* ITD, the test demonstrated 87.5% sensitivity and 99.9% specificity. Confirmatory testing to assess for *FLT3* ITD mutations is performed in parallel using PCR and fragment length analysis. The limit of detection of this test is 1% for the *JAK2* V617F and *NPM1* W288Cfs*12 variants and 5% for all other variants. Although VAF is provided as a percentage for DNA variants, this is not a quantitative test.

Based on validation, the RNA fusion testing demonstrated 95.7% accuracy and 100% specificity in gene fusion identification. The lower limit of detection is approximately 10% fusion supporting reads present in the submitted specimen. Fusions resulting from complex rearrangements and structural variants that do not lead to a chimeric fusion transcript may not be detected.

Tumor heterogeneity, tumor burden, specimen degradation or other limitations of the technology may affect the sensitivity and limit of detection, either broadly across the regions of interest or for specific regions, and may lead to false negative results.

This test does not detect copy number changes and does not distinguish between variants that are inherited versus acquired.

Chronic Myeloid Neoplasms Next-Generation Sequencing Panel

Overview

The Chronic Myeloid Neoplasms Next-Generation Sequencing Panel analyzes the clinically relevant regions of 56 genes known to be mutated in myeloid neoplasms. Performed on peripheral blood, bone marrow aspirate, FFPE tissues, and clot sections, this test identifies single nucleotide variants and small insertions and deletions in the targeted genes (refer to **Table 3**).

Clinical Indications

This assay is intended for molecular evaluation of known or suspected chronic myeloid neoplasms, including but not limited to myeloproliferative neoplasms, myelodysplastic syndromes, and overlap myelodysplastic/myeloproliferative neoplasms, to aid in the diagnosis, classification, prognosis, and/or therapeutic options of these entities.

Interpretation

Variants are classified according to established guidelines, and an interpretation is provided. Reported variants include those of strong or potential clinical significance and variants of unclear clinical significance. Common population variants are not included in the report.

Methodology

Nucleic acid (DNA), extracted from the specimen is subjected to anchored Multiplex PCR-based target enrichment and sequenced using Illumina (San Diego, CA) chemistry.

Based on validation, this DNA testing delivered an average of >500X coverage and >98% of targeted regions showed over 100X coverage. The test demonstrated 95.2% sensitivity and 99.9% specificity in identifying single nucleotide variants,

small insertions and deletions (indels) (less than 10bp) of >5% variant allele fraction (VAF). For the identification of large indels (greater than 10bp) >5% VAF, including *FLT3* ITD, the test demonstrated 87.5% sensitivity and 99.9% specificity. Confirmatory testing to assess for *FLT3* ITD mutations is performed in parallel using PCR and fragment length analysis. The limit of detection of this test is 1% for the *JAK2* V617F and *NPM1* W288Cfs*12 variants, and 5% for all other variants. Although VAF is provided as a percentage, this is not a quantitative test.

Tumor heterogeneity, tumor burden, specimen degradation, or other limitations of the technology may affect the sensitivity and limit of detection, either broadly across the regions of interest or for specific regions, and may lead to false negative results.

This test does not detect structural variants or copy number changes, and does not distinguish between variants that are inherited versus acquired.

Hematologic Neoplasm Fusion Next-Generation Sequencing Panel

Overview

The Hematologic Neoplasm Fusion Next-Generation Sequencing Panel analyzes the clinically relevant regions of 107 genes for RNA gene fusions or rearrangements that are known to be involved in hematologic neoplasms. Performed on peripheral blood, bone marrow aspirates, FFPE tissue and clot sections, this test identifies fusions in the targeted genes. (refer to **Table 2**)

For combined detection of RNA gene fusions and DNA somatic mutations (single nucleotide variants, small insertions, and deletions), please refer to the **Acute Leukemia Next-Generation Sequencing Panel.**

Clinical Indications

This test is intended for follow-up testing of peripheral blood under selected circumstances.

It is **NOT intended** for use in initial evaluation of known or suspected acute leukemias *(recommended test: Acute Leukemia NGS panel)*, chronic myeloid neoplasms *(recommended test: Chronic Myeloid NGS panel)*, eosinophilia related neoplasms *(recommended test: FISH for Myeloproliferative Neoplasms Panel)* or chronic myeloid leukemia *(recommended test: BCR-ABL Qualitative Multiplex RT-PCR)*.

This test may be useful in evaluation for these conditions as a follow-up when the initial recommended tests are inconclusive, clinically incongruent, or cannot be performed.

This test may also be performed by the laboratory for Acute Lymphoblastic Leukemias (ALLs) with insufficient specimen for combined DNA and RNA NGS testing.

Interpretation

Variants are classified according to established guidelines, and an interpretation is provided. Reported gene fusions or rearrangements include those of strong or potential clinical significance and variants of unclear clinical significance. Common population variants are not included in the report.

Methodology & Limitations

Nucleic acid (RNA), extracted from the specimen is subjected to anchored Multiplex PCR-based target enrichment and sequenced using Illumina (San Diego, CA) chemistry.

Based on validation, this test demonstrated 95.7% accuracy and 100% specificity in gene fusion identification. The lower limit of detection is approximately 10% fusion supporting reads present in the submitted specimen.

This test does not detect single nucleotide variants, small deletions, or copy number changes. Fusions resulting from complex rearrangements and structural variants that do not lead to a chimeric fusion transcript may not be detected. Tumor heterogeneity, tumor burden, specimen degradation or other limitations of the technology may affect the sensitivity and limit of detection, either broadly across the regions of interest or for specific regions, and may lead to false negative results.

Table 1: Acute Leukemia NGS Panel – DNA Targeted Gene Regions
DNA variants, 63 genes:

GENE	TRANSCRIPT	EXON(S) COVERED
ABL1	NM_005157.5	4–6
ASXL1	NM_15338.5	10–13
BCOR	NM_17745.5	2–15
BCORL1	NM_021946.4	1–12
BRAF	NM_004333.4	15
CALR	NM_004343.3	9
CBL	NM_005188.3	8–9
CDKN2A	NM_000077.4	1–2
CDKN2A	NM_058195.3	1
CEBPA	NM_004364.4	1
CSF3R	NM_000760.3	14–17
CUX1	NM_001202543.1	15–24
CUX1	NM_001913.4	1–23
DDX41	NM_016222.3	1–17
DNMT3A	NM_022552.4	2–23
EED	NM_003797.4	1–12
ETNK1	NM_018638.4	3
ETV6	NM_001987.4	1–8
EZH2	NM_004456.4	2–20
FBXW7	NM_018315.5	7–11
FLT3	NM_004119.2	14–17, 19–20
GATA1	NM_002049.3	2, 4
GATA2	NM_032638.4	2–6
GNAS	NM_000516.5	8–11
IDH1	NM_005896.3	4
IDH2	NM_002168.3	4
IKZF1	NM_006060.5	2–3, 5–7
JAK2	NM_004972.3	12–16
JAK3	NM_000215.3	11–18
KDM6A	NM_021140.3	1–29
KIT	NM_000222.2	2, 8–11, 13, 17
KMT2A	NM_005933.3	1–36
KRAS	NM_004985.4	2–4
LUC7L2 (C7orf55)	NM_001244585.1	2–11

GENE	TRANSCRIPT	EXON(S) COVERED
MPL	NM_005373.2	10–11
MYD88	NM_002468.4	5
NF1	NM_000267.3	1–57
NF1	NM_001042492.2	31
NOTCH1	NM_17617.4	26, 27, 34
NPM1	NM_002520.6	8–11
NRAS	NM_002524.4	2–4
PAX5	NM_016734.2	1–10
PHF6	NM_001015877.1	2–10
PIGA	NM_002641.3	2–6
PPM1D	NM_003620.3	1–6
PRPF8	NM_006445.3	2–43
PTEN	NM_000314.6	1–9
PTPN11	NM_002834.3	3, 4, 12, 13
RAD21	NM_006265.2	2–14
RIT1	NM_006912.5	5
RUNX1	NM_001754.4	2–9
RUNX1	NM_001122607.1	5
SETBP1	NM_015559.2	4*
SF3B1	NM_012433.3	13–16
SH2B3	NM_005475.2	2
SMC1A	NM_006306.3	1–25
SMC3	NM_005445.3	1–29
SRSF2	NM_003016.4	1–2
STAG2	NM_00104279.2	3–35
STAT3	NM_003150.3	20–21
STAT5B	NM_012448.3	16–18
SUZ12	NM_015355.3	1–16
TET2	NM_001127208.2	3–11
TP53	NM_000546.5	2–11
U2AF1	NM_006758.2	2, 6, 7
WT1	NM_000378.4	1–9
ZRSR2	NM_005089.3	1–11

**SETBP1*: Exon is only partially analyzed from genomic coordinates chr18:42531679-42532175.

Table 2: Acute Leukemia and Hematologic Neoplasm Fusion NGS Panels – RNA Targeted Gene Regions
RNA fusion variants, 107 genes:

GENE	TRANSCRIPT	EXON(S) COVERED
ABL1	NM_005157.5	1–11
ABL2	NM_005158.4	2–8
AFDN (MLLT4)	NM_001040000.2	2
ALK	NM_004304.4	2, 4, 6, 10, 16–23, 25
BCL11B	NM_138576.2	2, 3, 4
BCL2	NM_000633.2	1, 2, 3
BCL3	NM_005178.4	2–6
BCL6	NM_001706.4	2–5, 8, 9
BCR	NM_004327.3	1–3, 8, 12–16
BIRC3	NM_001165.4	4, 6, 7, 9
BLNK	NM_013314.3	1, 3–7, 12, 16, 17
CBFB	NM_022845.2	4, 5
CBL	NM_005188.3	2, 8
CCND1	NM_053056.2	1–5
CCND2	NM_001759.3	1–5
CCND3	NM_001760.4	2–5
CD274	NM_014143.3	2–5, 7
CD28	NM_006139.3	4
CDK6	NM_001259.6	1–4
CDKN2A	NM_000077.4	1, 2, 3
CEBPA	NM_004364.4	1
CEBPD	NM_005195.3	1
CEBPE	NM_001805.3	1, 2
CEBPG	NM_001806.3	2
CHD1	NM_001270.2	1, 2
CHIC2	NM_012110.3	1, 2, 3
CIITA	NM_000246.3	1, 2
CREBBP	NM_004380.2	2–6, 16, 20, 26, 30
CRLF2	NM_022148.4	1–6
CSF1R	NM_005211.3	9–14
CTLA4	NM_005214.4	1–4
DEK	NM_003472.3	2, 3
DGKH	NM_152910.5	2–10
DUSP22	NM_020185.4	1, 2
EBF1	NM_024007.4	10–15
EIF4A1	NM_001416.3	2, 3
EPOR	NM_000121.3	7, 8
ERG	NM_004449.4	7–11
ETV6	NM_001987.4	1–6
FGFR1	NM_023110.2	2–12, 17
FLT3	NM_004119.2	14–18
FOXP1	NM_032682.5	2, 3, 5, 6, 8, 12–15, 20, 21
GLIS2	NM_032575.2	2, 3
HLF	NM_002126.4	4
ID4	NM_001546.3	1, 2, 3
IKZF1	NM_006060.5	1, 2, 3, 7, 8
IKZF2	NM_016260.2	3, 4
IKZF3	NM_012481.4	2–7
IL2RB	NM_000878.4	2
IRF4	NM_002460.3	1, 3, 5, 7–9
IRF8	NM_002163.2	2, 3, 5, 7, 9
ITK	NM_005546.3	5–8
JAK2	NM_004972.3	6–22
KAT6A	NM_006766.4	13–17
KLF2	NM_016270.3	2, 3
KMT2A	NM_005933.3	2–35
LMO1	NM_002315.2	1–4
LMO2	NM_005574.3	3–6
LYN	NM_001111097.2	2–8
MALT1	NM_006785.3	9
MECOM	NM_004991.3	1–5, 8
MEF2D	NM_005920.3	3–7, 9
MLF1	NM_022443.4	2, 3, 4
MLLT10	NM_004641.3	2–18

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Table 2: Acute Leukemia and Hematologic Neoplasm Fusion NGS Panels – RNA Targeted Gene Regions (continued)

GENE	TRANSCRIPT	EXON(S) COVERED
MRTFA (MKL1)	NM_020831.4	4, 5, 6
MUC1	NM_002456.5	2, 3, 5, 7
MYC	NM_002467.4	1, 2, 3
MYH11	NM_002474.2	5–11, 14–20
NF1	NM_000267.3	14, 36
NFKB2	NM_002502.5	14–21
NOTCH1	NM_017617.4	24 – 29, 34
NTRK3	NM_002530.3, NM_001007156.2	4, 7, 10, 13–16 15
NUP214	NM_005085.3	17, 18, 19
NUP98	NM_016320.4	8–17
NUTM1	NM_175741.2	2(UTR) – 6
P2RY8	NM_178129.4	1
PAG1	NM_018440.3	2
PAX5	NM_016734.2	1, 3–8
PBX1	NM_002585.3	1–9
PDCD1	NM_005018.2	1, 2, 3, 5
PDCD1LG2	NM_025239.3	1, 2, 3, 5, 6
PDGFRA	NM_006206.4	9–15, 18
PDGFRB	NM_002609.3	8–14
PICALM	NM_007166.3	15, 17, 18, 19
PML	NM_002675.3	2–7

GENE	TRANSCRIPT	EXON(S) COVERED
PRDM16	NM_022114.3	1–4
PTK2B	NM_173176.2	2–8
RARA	NM_000964.3	1–9
RBM15	NM_022768.4	1
ROS1	NM_002944.2	31–38
RUNX1	NM_001754.4	1–9
RUNX1T1	NM_001198679.1	1–9
SEMA6A	NM_020796.4	1, 2
SETD2	NM_014159.6	1, 3–12
STIL	NM_003035.2	1, 2
SYK	NM_003177.6	5–8
TAL1	NM_003189.5, NM_001290404.1	2–6 2
TCF3	NM_003200.3	11–18
TFG	NM_006070.5	2, 3, 4
TLX1	NM_005521.4	1, 2, 3
TLX3	NM_021025.3	1, 2, 3
TP63	NM_003722.4	3, 4, 5
TSLP	NM_033035.4	1–4
TYK2	NM_003331.4	7, 8, 16, 18
VAV1	NM_005428.3	25
ZCCHC7	NM_032226.2	1, 2, 4
ZNF384	NM_001135734.2	2–9

Table 3: Chronic Myeloid Neoplasms NGS Panel – DNA Targeted Gene Regions
DNA variants, 56 genes:

GENE	TRANSCRIPT	EXON(S) COVERED
ABL1	NM_005157.5	4–6
ASXL1	NM_15338.5	10–13
BCOR	NM_17745.5	2–15
BCORL1	NM_021946.4	1–12
BRAF	NM_004333.4	15
CALR	NM_004343.3	9
CBL	NM_005188.3	8–9
CEBPA	NM_004364.4	1
CSF3R	NM_000760.3	14–17
CUX1	NM_001202543.1 NM_001913.4	15–24 1–23
DDX41	NM_016222.3	1–17
DNMT3A	NM_022552.4	2–23
EED	NM_003797.4	1–12
ETNK1	NM_018638.4	3
ETV6	NM_001987.4	1–8
EZH2	NM_004456.4	2–20
FLT3	NM_004119.2	14–17, 19–20
GATA1	NM_002049.3	2, 4
GATA2	NM_032638.4	2–6
IDH1	NM_005896.3	4
IDH2	NM_002168.3	4
JAK2	NM_004972.3	12–16
JAK3	NM_000215.3	11–18
KDM6A	NM_021140.3	1–29
KIT	NM_000222.2	2, 8–11, 13, 17
KMT2A	NM_005933.3	1–36
KRAS	NM_004985.4	2–4
LUC7L2 (C7orf55)	NM_001244585.1	2–11

GENE	TRANSCRIPT	EXON(S) COVERED
MPL	NM_005373.2	10–11
NF1	NM_000267.3 NM_001042492.2	1–57 31
NPM1	NM_002520.6	8–11
NRAS	NM_002524.4	2–4
PHF6	NM_001015877.1	2–10
PIGA	NM_002641.3	2–6
PPM1D	NM_003620.3	1–6
PRPF8	NM_006445.3	2–43
PTEN	NM_000314.6	1–9
PTPN11	NM_002834.3	3, 4, 12, 13
RAD21	NM_006265.2	2–14
RIT1	NM_006912.5	5
RUNX1	NM_001754.4 NM_001122607.1	2–9 5
SETBP1	NM_015559.2	4*
SF3B1	NM_012433.3	13–16
SH2B3	NM_005475.2	2
SMC1A	NM_006306.3	1–25
SMC3	NM_005445.3	1–29
SRSF2	NM_003016.4	1–2
STAG2	NM_00104279.2	3–35
STAT3	NM_003150.3	20–21
STAT5B	NM_012448.3	16–18
SUZ12	NM_015355.3	1–16
TET2	NM_001127208.2	3–11
TP53	NM_000546.5	2–11
U2AF1	NM_006758.2	2, 6, 7
WT1	NM_000378.4	1–9
ZRSR2	NM_005089.3	1–11

**SETBP1:* Exon is only partially analyzed from genomic coordinates chr18:42531679-42532175.

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Test Overviews

Test Name	**Acute Leukemia** Next Generation Sequencing Panel	
Test Targets	**DNA variants** (63-gene) **& RNA fusion variants** (107-gene)	
Order Code(s)	HDMNGS (Bone Marrow) HDPNGS (Peripheral Blood) HDONGS (Other, FFPE, Clot sections)	
Methodology	Next-Generation Sequencing (NGS)	
Specimen Requirements	**Bone Marrow Aspirate:** 8 mL, EDTA (lavender) **Peripheral Blood:** 8 mL, EDTA (lavender) **FFPE:** 10 charged, unbaked, unstained FFPE slides; or, two (2) 10 x 7 micron FFPE curls (scrolls) plus 1 H&E slide with best tumor area circled containing at least 8% tumor	
Stability	**Bone Marrow or Peripheral Blood** Ambient: 48 hours Refrigerated: 3 days Frozen: Unacceptable	**FFPE** Ambient: Indefinitely Refrigerated: Indefinitely Frozen: Unacceptable
Days Performed	2–3 days per week	
Days Reported	10 calendar days	
CPT Code	81455	

Test Name	**Chronic Myeloid Neoplasms** Next Generation Sequencing Panel	
Test Targets	**DNA variants** (56-gene)	
Order Code(s)	MYNGSM (Bone marrow) MYNGSP (Peripheral blood) MYNGSO (Other, FFPE, Clot sections)	
Methodology	Next-Generation Sequencing (NGS)	
Specimen Requirements	**Bone Marrow Aspirate:** 4 mL, EDTA (lavender) **Peripheral Blood:** 4 mL, EDTA (lavender) **FFPE:** 10 charged, unbaked, unstained FFPE slides; or, 10 x 7 micron FFPE curls (scrolls) plus 1 H&E slide with best tumor area circled containing at least 8% tumor	
Stability	**Bone Marrow or Peripheral Blood** Ambient: 48 hours Refrigerated: 7 days Frozen: Unacceptable	**FFPE** Ambient: Indefinitely Refrigerated: Indefinitely Frozen: Unacceptable
Days Performed	2–3 days per week	
Days Reported	10 calendar days	
CPT Code	81455	

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Test Overview

Test Name	**Hematologic Neoplasm Fusion** Next Generation Sequencing Panel	
Test Targets	**RNA fusion variants** (107-gene)	
Order Code(s)	HFMNGS (Bone Marrow) HFPNGS (Peripheral Blood) HFONGS (Other, FFPE, Clot sections)	
Methodology	Next-Generation Sequencing (NGS)	
Specimen Requirements	**Bone Marrow Aspirate:** 4 mL, EDTA (lavender) **Peripheral Blood:** 4 mL, EDTA (lavender) **FFPE:** 10 charged, unbaked, unstained FFPE slides; or, 10 x 7 micron FFPE curls (scrolls) plus 1 H&E slide with best tumor area circled containing at least 8% tumor	
Stability	**Bone Marrow or Peripheral Blood** Ambient: 48 hours Refrigerated: 3 days Frozen: Unacceptable	**FFPE** Ambient: Indefinitely Refrigerated: Indefinitely Frozen: Unacceptable
Days Performed	2–3 days per week	
Days Reported	10 calendar days	
CPT Code	81455	

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