

INFORMATION ASSAYS HEMATO-ONCOLOGY LAB - UNIT MOLECULAR DIAGNOSTICS EMC – FUSION GENES (RNA) AND MUTATIONS/SEQUENCING (DNA)

Abnormality	Percentage Malignant Cells*	VAF#	Assay
<i>RUNX1::RUNX1T1</i>	5%/ 0.01%		RT-PCR > LMA/ RQ-PCR1
<i>CBFB::MYH11</i>	5%		RT-PCR > LMA
<i>DEK::NUP214</i>	5%		RT-PCR > LMA
<i>KMT2A::ELL</i>	5%		RT-PCR > LMA
<i>KMT2A::MLLT3</i>	5%		RT-PCR > LMA
<i>KMT2A::MLLT4</i>	5%		RT-PCR > LMA
<i>NPM1::MLF1</i>	5%		RT-PCR > LMA
<i>BCR::ABL</i> (breakpoint)	1%/ 5%		RT-PCR/ RT-PCR > LMA
<i>BCR::ABL</i> (e13/e14-a2)	0.001%		RQ-PCR2
<i>BCR::ABL</i> mutation	>0.1% IS		RT-PCR > NGS
<i>NUP214::ABL</i>	0.1%		RQ-PCR1
<i>FIP1L1::PDGFRA</i>	1%		RT-PCR
<i>PML::RARA</i>	1%/ 5%		RT-PCR/ RT-PCR > LMA
<i>MLL::AF4</i>	1%		RT-PCR
<i>FLT3</i> ITD	10%	0.05 (ratio)^	PCR > LMA
<i>FLT3</i> TKD	10%	0.05 (ratio)^	PCR > LMA
<i>ASXL1</i> mutation	10%	5%	NGS1
<i>BCOR</i> mutation	10%	5%	NGS1
<i>BCORL1</i> mutation	10%	5%	NGS1
<i>BRAF</i> mutation	10%	5%	NGS1
<i>CALR</i> mutation	10%	5%	NGS1 or NGS4
<i>CBL</i> mutation	10%	5%	NGS1
<i>CEBPA</i> mutation	10%	5%	NGS2
<i>CSF3R</i> mutation	10%	5%	NGS1 en NGS2
<i>DDX41</i> mutation	10%	5%	NGS1
<i>DNMT3A</i> mutation	10%	5%	NGS1
<i>ETNK1</i> mutation	10%	5%	NGS1
<i>ETV6</i> mutation	10%	5%	NGS1
<i>EZH2</i> mutation	10%	5%	NGS1
<i>GATA2</i> mutation	10%	5%	NGS1
<i>IDH1</i> mutation [screening]	5%	1%	allele-specific PCR
<i>IDH1</i> mutation	10%	5%	NGS1
<i>IDH2</i> mutation [screening]	5%	1%	allele-specific PCR
<i>IDH2</i> mutation	10%	5%	NGS1
<i>JAK2</i> V617F mutation	0.1%	2%	RQ-PCR1/NGS4 or NGS1
<i>JAK2</i> mutation	10%	5%	NGS1 or NGS4
<i>KIT</i> mutation	10%	5%	NGS1
<i>MPL</i> mutation	10%	5%	NGS1 or NGS4
<i>MYD88</i> mutation	10%	5%	NGS1
<i>NF1</i> mutation	10%	5%	NGS1
<i>NPM1</i> mutation [screening]	5%	1%	locked nucleic acid LNA-PCR
<i>NPM1</i> mutation	0.01% (RQ-PCR*)	1% (NGS)	RQ-PCR1/NGS1
<i>NRAS</i> mutation	10%	5%	NGS1
<i>KRAS</i> mutation	10%	5%	NGS1
<i>PHF6</i> mutation	10%	5%	NGS1
<i>PPM1D</i> mutation	10%	5%	NGS1
<i>PTPN11</i> mutation	10%	5%	NGS1
<i>RUNX1</i> mutation	10%	5%	NGS1
<i>SETBP1</i> mutation	10%	5%	NGS1
<i>SF3B1</i> mutation	10%	5%	NGS1
<i>SRSF2</i> mutation	10%	5%	NGS1
<i>STAG2</i> mutation	10%	5%	NGS1
<i>TET2</i> mutation	10%	5%	NGS1
<i>TP53</i> mutation	10%	5%	NGS1/NGS3
<i>U2AF1</i> mutation	10%	5%	NGS1
<i>UBA1</i> mutation	10%	5%	NGS5
<i>ZRSR2</i> mutation	10%	5%	NGS1
Factor V Leiden	NA	NA	RQ-PCR2
Prothrombine	NA	NA	RQ-PCR2

*De result not detectable in case of AML is only reliable above the indicated percentage of malignant cells or VAF
#VAF: variant allele frequency (e.g. VAF 50% means that all the cells carry a heterozygous mutation) – VAF below the percentage indicated are also reported

*The *FLT3* ITD and *FLT3* TKD mutation results are reported as ratio (*FLT3* ITD/ *FLT3* wild type of *FLT3* TKD/ *FLT3* wild type) according to ELN 2017 (no VAF)

*NPM1 mutant type A, B and D

PCR: polymerase chain reaction

RT-PCR: reverse transcriptase – PCR

RT-PCR/LMA: polymerase chain reaction > length mutation analysis (Biotype Mentype AMLplex)

PCR>LMA: polymerase chain reaction > length mutation analysis

RQ-PCR1: real-time quantitative – PCR (in-house test)

RQ-PCR2: real-time quantitative – PCR (Cepheid)

RT-PCR > NGS: reverse-transcriptase > next generation sequencing *BCR::ABL1*

NGS1: Next generation sequencing Illumina Pillar oncoReveal Myeloid Panel

NGS2: Next generation sequencing custom panel *CEBPA CEBPA en CSF3R* (exon18-19)

NGS3: Next generation sequencing custom panel *TP53*

NGS4: Next generation sequencing custom panel *JAK2 V617F, JAK2 exon 12, CALR, MPL*

NGS5: Next generation sequencing custom panel *UBA1*

Below the markers determined in the Hemato-oncology laboratory unit Molecular Diagnostics are indicated per malignancy.

Disclaimer next generation sequencing:

Neutral polymorphisms, silent mutations, likely not-pathogenic variants and variants, that are currently unclassifiable, will not be reported. The method is limited for the detection and/or exclusion of mutations below a variant allele frequency of 5% as well as (larger) deletions, insertions and indels. Some parts of genes are more difficult to sequence such that mutations may be missed.

Markers Hemato-oncology laboratory unit Molecular Diagnostics ranked by malignancy

AML

RUNX1::RUNX1T1
CBFB::MYH11
DEK::NUP214
KMT2A::ELL
KMT2A::MLLT3
KMT2A::MLLT4
NPM1::MLF1
BCR::ABL1
PML::RARA
ASXL1 mutation
BCOR mutation
CALR mutation
CEBPA mutation (incl. presence/ absence in frame bZIP mutation)
DDX41 mutation
EZH2 mutation
FLT3-ITD
FLT3-TKD
GATA2 mutation
IDH1 mutation
IDH2 mutation
JAK2 mutation
KIT mutation
KRAS mutation
MPL mutation
NPM1 mutation
NRAS mutation
RUNX1 mutation
SF3B1 mutation
SRSF2 mutation
STAG2 mutation
TET2 mutation
TP53 mutation
U2AF1 mutation
ZRSR2 mutation

Type mutations/ regio

NM_015338.6: exon12 (exon13 partial)
NM_001123385.2: exons3-14
NM_004343.4: exon9
NM_004364.5: exon1
NM_016222.4: exons1,3,5,6,8,10,11,14,15
NM_004456.5: exons2-19 (exon20 partial)
NM_004119.3: exon14 en exon15
NM_004119.3: exon20 (D835)
NM_032638.5: exons3-5 (exon2, 6 partial)
NM_005896.4: exon4 (R132)
NM_002168.4: exons4,6 (R140 en R172)
NM_004972.4: exons12-15
NM_000222.3: exon2,8,9,10,11,13,14,15,17,18
NM_004985.5: exon2-4
NM_005373.3: exon10 (W515)
NM_002520.7: exon11 (partial)
NM_002524.5: exons3, 4 (exon2 partial)
NM_001754.5: exons2-8 (exon9 partial)
NM_012433.4: exon13-16 (K700)
NM_001195427.2: exon1 (partial)
NM_001042750.2: exons4-34 (exons3, 35 partial)
NM_001127208.3: exon4-10 (exon3, 11 partial)
NM_000546.6: exon2-10 (exon11 partial)
NM_006758.3: exon2-6 (exon1, 8 partial)
NM_005089.4: exon1-11

MDS (IPSS-M)

ASXL1 mutation
BCOR mutation
BCORL1 mutation
CALR mutation
CBL mutation
CEBPA mutation (inclusief aan-/afwezigheid bZIP mutation)
CSF3R mutation
DDX41 mutation
DNMT3A mutation
ETNK1 mutation
ETV6 mutation
EZH2 mutation
FLT3-ITD
FLT3-TKD
GATA2 mutation
IDH1 mutation
IDH2 mutation
JAK2 mutation
KIT mutation
KRAS mutation
NF1 mutation
NPM1 mutation
NRAS mutation
MPL mutation
PHF6 mutation
RUNX1 mutation
PPM1D mutation
PTPN11 mutation
SETBP1 mutation
SF3B1 mutation
SRSF2 mutation
STAG2 mutation
TET2 mutation
TP53 mutation
U2AF1 mutation
WT1 mutation
ZRSR2 mutation

NM_015338.6: exon12 (exon13 partial)
NM_001123385.2: exons3-14
NM_001379451.1: exons3-13 (exons2,14 partial)
NM_004343.4: exon9
NM_005188.4: exons3,5,8,9,10,12,13 (exons1,2,16 partial)
NM_004364.5: exon1
NM_000760.4: exons14-18
NM_016222.4: exons1,3,5,6,8,10,11,14,15
NM_022552.5: exons3-22 (exons2, 23 partial)
NM_018638.5: exon3
NM_022552.5: exons2-7 (exons1, 8 partial)
NM_004456.5: exons2-19 (exon20 partial)
NM_004119.3: exon14 en exon15
NM_004119.3: exon20 (D835)
NM_032638.5: exons3-5 (exon2, 6 partial)
NM_005896.4: exon4 (R132)
NM_002168.4: exons4,6 (R140 en R172)
NM_004972.4: exons12-15
NM_000222.3: exon2,8,9,10,11,13,14,15,17,18
NM_004985.5: exon2-4
NM_001042492.3: exons2,3,6,12,30,41,45,49,52 (exons1,4,37,38,39,58 partial)
NM_002520.7: exon11 (partial)
NM_002524.5: exons3, 4 (exon2 partial)
NM_005373.3: exon10 (W515)
NM_001015877.2: exons3-10 (exon2 partial)
NM_001754.5: exons2-8 (exon9 partial)
NM_003620.4: exon6 (partial)
NM_002834.5: exons3, 13
NM_015559.3: exon4
NM_012433.4: exon13-16 (K700)
NM_001195427.2: exon1 (partial)
NM_001042750.2: exons4-34 (exons3, 35 partial)
NM_001127208.3: exon4-10 (exon3, 11 partial)
NM_000546.6: exon2-10 (exon11 partial)
NM_006758.3: exon2-6 (exon1, 8 partial)
NM_024426.6: 2-9 (exons1, 10 partial)
NM_005089.4: exon1-11

NPM1 mutation (follow up)

Mutation type A, B en D

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MDS-MPN/ CMML/ CNL en atypische CML

ASXL1 mutation	NM_015338.6: exon12 (exon13 partial)
BCOR mutation	NM_001123385.2: exons3-14
BCORL1 mutation	NM_001379451.1: exons3-13 (exons2,14 partial)
CALR mutation	NM_004343.4: exon9
CBL mutation	NM_005188.4: exons3,5,8,9,10,12,13 (exons1,2,16 partial)
CEBPA mutation (inclusief aan-/afwezigheid bZIP mutation)	NM_004364.5: exon1
CSF3R mutation	NM_000760.4: exons14-18
DDX41 mutation	NM_016222.4: exons1,3,5,6,8,10,11,14,15
DNMT3A mutation	NM_022552.5: exons3-22 (exons2, 23 partial)
ETNK1 mutation	NM_018638.5: exon3
ETV6 mutation	NM_022552.5: exons2-7 (exons1, 8 partial)
EZH2 mutation	NM_004456.5: exons2-19 (exon20 partial)
GATA2 mutation	NM_032638.5: exons3-5 (exon2, 6 partial)
IDH1 mutation	NM_005896.4: exon4 (incl. R132)
IDH2 mutation	NM_002168.4: exons4,6 (incl. R140 en R172)
JAK2 mutation	NM_004972.4: exons12-15
KIT mutation	NM_000222.3: exon2,8,9,10,11,13,14,15,17,18
KRAS mutation	NM_004985.5: exon2-4
NF1 mutation	NM_001042492.3: exons2,3,6,12,30,41,45,49,52 (exons1,4,37,38,39,58 partial)
NPM1 mutation	NM_002520.7: exon11 (partial)
NRAS mutation	NM_002524.5: exons3, 4 (exon2 partial)
MPL mutation	NM_005373.3: exon10 (incl. W515)
PHF6 mutation	NM_001015877.2: exons3-10 (exon2 partial)
RUNX1 mutation	NM_001754.5: exons2-8 (exon9 partial)
PPM1D mutation	NM_003620.4: exon6 (partial)
PTPN11 mutation	NM_002834.5: exons3, 13
SETBP1 mutation	NM_015559.3: exon4
SF3B1 mutation	NM_012433.4: exon13-16 (incl. K700)
SRSF2 mutation	NM_001195427.2: exon1 (partial)
STAG2 mutation	NM_001042750.2: exons4-34 (exons3, 35 partial)
TET2 mutation	NM_001127208.3: exon4-10 (exon3, 11 partial)
TP53 mutation	NM_000546.6: exon2-10 (exon11 partial)
U2AF1 mutation	NM_006758.3: exon2-6 (exon1, 8 partial)
WT1 mutation	NM_024426.6: 2-9 (exons1, 10 partial)
ZRSR2 mutation	NM_005089.4: exon1-11

MDS-RS

SF3B1 mutation	NM_012433.4: exon13-16 (incl. K700)
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MPN

CALR mutation	NM_004343.4: exon9
JAK2 mutation	NM_004972.4: exons12-15
MPL mutation	NM_005373.3: exon10 (incl. W515)

PMF (MIPSS70)

ASXL1 mutation	NM_015338.6: exon12 (exon13 partial)
CALR mutation	NM_004343.4: exon9
EZH2 mutation	NM_004456.5: exons2-19 (exon20 partial)
IDH1 mutation	NM_005896.4: exon4 (incl. R132)
IDH2 mutation	NM_002168.4: exons4,6 (incl. R140 en R172)
JAK2 mutation	NM_004972.4: exons12-15
MPL mutation	NM_005373.3: exon10 (incl. W515)
RUNX1 mutation	NM_001754.5: exons2-8 (exon9 partial)
SF3B1 mutation	NM_012433.4: exon13-16 (incl. K700)
SRSF2 mutation	NM_001195427.2: exon1 (partial)
TP53 mutation	NM_000546.6: exon2-10 (exon11 partial)
U2AF1 mutation	NM_006758.3: exon2-6 (exon1, 8 partial)

CLL

TP53 mutation	NM_000546.6: exon2-11
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Hairy cell leukemie

BRAF mutation	NM_004333.6: exons11, 15 (incl. V600)
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Mastocytose

KIT mutation	NM_000222.3: exon2,8,9,10,11,13,14,15,17,18
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Agressieve mastocytose

ASXL1 mutation	NM_015338.6: exon12 (exon13 partial)
EZH2 mutation	NM_004456.5: exons2-19 (exon20 partial)
RUNX1 mutation	NM_001754.5: exons2-8 (exon9 partial)
SRSF2 mutation	NM_001195427.2: exon1 (partial)
TET2 mutation	NM_001127208.3: exon4-10 (exon3, 11 partial)

M. Waldenström

MYD88 mutation	NM_002468.5: exon3-5 (incl. L265)
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VEXAS

UBA1 mutation	NM_003334.4: exon3, 14, 16
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APL

PML::RARA (diagnosis and follow up)	BCR1-2 en BCR3
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CML

BCR::ABL1

all BCR::ABL1 breakpoints (diagnosis)
BCR::ABL1 e1a2, e13a2, e14a2, e18a2 and e19a2 (follow up)
BCR::ABL1 mutations (amino acid ~30-540aa (NP_005148.2))

ALL

BCR::ABL1

BCR::ABL1 e1a2, e13a2, e14a2, e18a2 and e19a2 (follow up)
BCR::ABL1 mutations (amino acid ~30-540aa (NP_005148.2))

B-ALL: RNASeq analyses (see below)

Predisposition gene panel

information: <https://hema13.erasmusmc.nl/diagnostiek.html>

Assay description NGS (DNA)

The variants in the mentioned genes are determined using Next Generation Sequencing (NGS) on DNA, using the Pillar Myeloid Panel or custom gene panels on the Illumina MiSeq platform¹. The coverage of all target genes is at least 500x. For the exon-intron junctions, 5 base pairs (bp) are analysed.

Based on this NGS data (paired-end reads), variants are identified using an in-house developed bioinformatics pipeline. This pipeline starts with the alignment of the reads to the reference genome using the BMAP tool². Per patient, only the regions containing the specified genes are analysed. For this, an in-house developed tool is used that filters or trims reads falling outside these regions, while simultaneously removing adapters. For the detection of single nucleotide variants (SNVs), insertions, and deletions (indels), the variant callers Mutect³, Mutect2⁴, VarDict⁵ and Strelka2⁶ are used. For the detection of larger indels, such as FLT3-ITDs and variants in the CEBPA gene, the tool Pindel⁷ is additionally used. The variants are annotated using the tools ANNOVAR⁸, SNPEff⁹, and an internal tool for determining coverage and variant allele frequency (VAF). The result is a list of annotated variants per patient.

All analyses are carried out on two independent DNA samples. Only the variants present in both samples are reported. If variants are detected in DNA derived from bone marrow or blood at the time of malignancy, they are confirmed through analysis of DNA obtained from saliva.

To assess the potential pathogenicity of the variants, several in silico prediction programs are used, including SIFT¹⁰, PolyPhen¹¹, MutationTaster¹², Franklin (<https://franklin.genoox.com>), and the Genome Aggregation Database (gnomAD).

	Part of gene
chr21:36265116-36265292	RUNX1
chrX:123184867-123185039	STAG2
chrX:123195585-123195750	STAG2
chrX:123197732-123197921	STAG2
chrX:123199965-123200094	STAG2
chrX:123204971-123205146	STAG2
chrX:123215319-123215488	STAG2
chrX:123217200-123217378	STAG2
chrX:133511947-133512112	PHF6
chrX:133527566-133527744	PHF6

References

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INFORMATION ASSAYS HEMATO-ONCOLOGY LAB - UNIT MOLECULAR DIAGNOSTICS EMC – SEQUENCING (RNA)

RNASeq analyses acute lymphoblastic leukemia (ALL)

This RNASeq analysis has not yet been incorporated into the ISO15189 (M138) scope.

Abnormality	Assay
<i>ETV6::RUNX1</i>	RNASeq*
High hyperdiploid (51-67 chr)	RNASeq*
<i>ZNF384</i> fusion genes	RNASeq*
<i>BCR::ABL1</i>	RNASeq*
<i>ABL</i> -class fusion genes (bijv. <i>ABL1</i> , <i>ABL2</i> , <i>CSF1R</i> and <i>PDGFRB</i> fusions)	RNASeq*
Low hypodiploid (30-39 chr)	RNASeq*
<i>TP53</i> mutation (homozygous-heterozygous)	RNASeq
<i>FLT3</i> ITD	RNASeq
<i>KMT2A::AFF1</i>	RNASeq*
<i>KMT2A</i> fusie genes	RNASeq*
Nearly triploid (60-78 chr)	RNASeq*
<i>CRLF2</i> - of <i>JAK2</i> -related fusion genes	RNASeq*
<i>JAK</i> mutations	RNASeq
<i>iAMP21</i>	RNASeq*
Complex karyotype (≥5)	RNASeq*
<i>CDKN2A/B</i> deletion	RNASeq*
<i>PAX5</i> deletion	RNASeq [#]
<i>PAR1</i> deletion	RNASeq [#]
<i>ERG</i> deletion	RNASeq [#]
<i>IKZF1</i> deletion	RNASeq [#]

Percentage malignant cells: >10%

*: in combination with cytogenetics

#: in combination with array CGH

Test description NGS (RNA)

The variants in as indicated above are detected by next generation sequencing on RNA using the KAPA RNA Hyper+RiboErase HMR (24rxn) (8098131702) reaction kit followed by analyses using our inhouse pipeline. Our pipeline for comprehensive profiling of RNA-seq data is implemented in Nextflow¹ for robust reproducibility and scalable execution in diverse computational environments. Briefly, RNA-seq reads are trimmed with TrimGalore (0.6.10)² and aligned to the GRCh38 human reference genome using STAR (2.7.11a)³. Next, they are processed with an in-house pipeline partially based on the GATK best practices, aimed at correcting possible sources of artifacts⁴. Point mutations and small inserts or deletions are called with an ensemble of tools, namely Mutect2⁵, VarDict⁶ and Pindel⁷. Fusion genes are detected with FusionCatcher (v1.3)⁸, Arriba (v2.2.21)⁹ and STAR-Fusion¹⁰, and copy number alterations (CNA) with SuperFreq (v1.3.2)¹¹. Other structural variants, such as large deletions, are identified by an in-house algorithm that extracts anomalous splicing junctions absent in the Ensembl database (v104)¹².

References

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Disclaimer next generation sequencing:

Neutral polymorphisms, silent mutations, likely not-pathogenic variants and variants, that are currently unclassifiable, will not be reported. The method is limited for the detection and/or exclusion of mutations below a variant allele frequency of 5% as well as (larger) deletions, insertions and indels. Some parts of genes are more difficult to sequence such that mutations may be missed.