ErasmusMC Universitair Medisch Centrum Rotterdam Kanker Instituut

INFORMATION ASSAYS HEMATO-ONCOLOGY LAB - UNIT MOLECULAR DIAGNOSTICS EMC

Abnormality	Percentage Malignant Cells*	VAF#	Assay
RUNX1-RUNX1T1	1%		RQ-PCR1
CBFB-MYH11	10%		RQ-PCR1/RT-PCR
FLT3 ITD	10%	0.05 (ratio)^	PCR > LMA
FLT3 TKD	10%	0.05 (ratio)^	PCR > LMA
NPM1 mutation	0.01% (RQ-PCR≠)	1% (NGS)	RQ-PCR1/NGS1
CEBPA mutation	10%	5%	NGS2
ASXL1 mutation	10%	5%	NGS1
TP53 mutation	10%	5%	NGS1/NGS3
RUNX1 mutation	10%	5%	NGS1
KIT mutation	10%	5%	NGS1
IDH1 mutation	10%	5%	NGS1
IDH2 mutation	10%	5%	NGS1
PML-RARA	1%		RT-PCR
MLL-AF4	1%		RT-PCR
BCR-ABL (breakpoint)	1%		RT-PCR
BCR-ABL (e13/e14-a2)	0.001%		RQ-PCR2
BCR-ABL mutation	only >0.01%		Sanger sequencing
NUP214-ABL	0.1%		RQ-PCR1
FIP1L1-PDGFRA	1%		RT-PCR
SETBP1 mutation	10%	5%	NGS1
NRAS mutation	10%	5%	NGS1
CSF3R mutation	10%	5%	NGS1
BRAF mutation	10%	5%	NGS1
SF3B1 mutation	10%	5%	NGS1
MYD88 mutation	10%	5%	NGS1
JAK2 V617F mutation	1%	2%	RQ-PCR1/NGS4
JAK2 exon12 mutation	10%	5%	NGS4
MPL mutation	10%	5%	NGS4
CALR mutation	10%	5%	NGS4
NRAS mutation	10%	5%	NGS1
EZH2 mutation	10%	5%	NGS1
U2AF1 mutation	10%	5%	NGS1
CBL mutation	10%	5%	NGS1
SRSF2 mutation	10%	5%	NGS1
TET2 mutation	10%	5%	NGS1
UBA1 mutation	10%	5%	NGS5

*De result not detectable in case of AML is only reliable above the indicated percentage of malignant cells or VAF
#VAF: variant allel frequency (eg. 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
RQ-PCR1: real-time quantitative – PCR (in-house test)
RQ-PCR2: real-time quantitative – PCR (Cepheid)
LMA: length mutation analyse
NGS1: Next generation sequencing Illumina Trusight Myeloid panel
NGS2: Next generation sequencing custom panel *CEBPA*
NGS3: Next generation sequencing custom panel *TP53*
NGS4: Next generation sequencing custom panel *JAK2, CALR, MPL*
NGS4: Next generation sequencing custom panel *UBA1*

On the next page 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.

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Markers Hemato-oncology laboratory unit Molecular Diagnostics ranked by malignancy

AML and MDS-EB	**Type mutations/ region**
RUNX1-RUNX1T1	
CBFB-MYH11	
NPM1 mutation	4bp insertie/ exon12
CEBPA mutation	complete gene
FLT3-ITD	exon14 en exon15
FLT3-TKD	D835/ exon20
ASXL1 mutation	exon12
RUNX1 mutation	complete gene
TP53 mutation	exon2-11
KIT mutation	exon2,8-11,13,17
IDH1 mutation	R132/ exon4
IDH2 mutation	R140 en R172/ exon4
CLL	
TP53 mutation	exon2-11
CMML	
ASXL1 mutation	exon12
SETBP1 mutation	(part of) exon4
NRAS mutation	codons 12,13 en 61/ exon2 en exon3
RUNX1 mutation	gehele gen
CNL and atypical CML	
ASXL1 mutation	exon12
CSF3R mutation	exon14-17
SETBP1 mutation	(part of) exon4
Hairy cell leukemia	
BRAF mutation	V600/ exon15
Mastocytosis	
KIT mutation	exon2,8-11,13,17
MPN	
CALR mutation	exon9
JAK2 mutation	V617F en exon12
MPL mutation	W515/ exon10
M. Waldenström	
MYD88 mutation	L265/ exon3-5
MDS	
SF3B1 mutation	K700/ exon13-16
EZH2 mutation	complete gene
U2AF1 mutation	exon2 en exon6
ASXL1 mutation	exon12
RUNX1 mutation	complete gene
TP53 mutation	exon2-11
CBL mutation	exon8 en exon9
MDS-RS	
SF3B1 mutation	K700/ exon13-16
VEXAS	
UBA1 mutation	exon3
Predispositie genpanel (uitsluitend na overleg)	informatie: https://hema13.erasmusmc.nl/diagnostiek.html

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Assay description

The variants in the genes indicated are determined by next generation sequencing using the Illumina TruSight Myeloid panel (TSCA design (probe hybridisatie, extensie-ligatie en PCR)) or custom panels (nested PCR) on the Illumina MiSeq[1]. The coverage of all target genes is at least 500x. Of the exon-intron boundaries 5bp is analysed. The variants are identified with an in-house developed software pipeline. In this software read alignment is done with BBMAP[2] and SAMtools[3], whereas 'variant calling' with MuTect[4], GATK[5], Varscan[6], Indellocator[7] and Pindel[5]. All analyses are carried out on two independent DNA samples. Only the DNA variants present in both samples are reported. To classify the varaints as pathogenic several *in silico* programs, such as SIFT[8], Polyphen[9] en Mutation Taster[10], Franklin [https://franklin.genoox.com] en de Genome Aggregation Database (gnomAD) are used.

Genomic regions with poorer coverage	Part of the following gene
chr1:115256418-115256622	*NRAS*
chr1:36932117-36932335	*CSF3R*
chr21:36259341-36259561	*RUNX1*
chr3:128200640-128200861	*GATA2*
chr3:128204781-128204979	*GATA2*

References

1. https://emea.illumina.com/.
2. BBMap short-read aligner, and other bioinformatics tools (http://sourceforge.net/projects/bbmap/).
3. Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078-9.
4. Cibulskis K, Lawrence MS, Carter SL, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. Nat Biotechnol 2013;31:213-9.
5. DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet 2011;43:491-8.
6. Koboldt DC, Zhang Q, Larson DE, et al. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res 2012;22:568-76.
7. Indelocator. at http://www.broadinstitute.org/cancer/cga/indelocator.
8. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res. 2003;31(13):3812-4.
9. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet. 2013;Chapter 7:Unit7.20.
10. http://www.mutationtaster.org/.

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