

Liquid Trace Hematology

Patient Name:	<input type="text"/>	Ordering Physician:	M.D. <input type="text"/>
Date of Birth:	<input type="text"/>	Physician ID:	<input type="text"/>
Gender (M/F):	<input type="text"/>	Accession #:	<input type="text"/>
Client:	<input type="text"/>	Specimen Type:	Peripheral Blood
Case #:	NGSXX-XXXXXX	Specimen ID:	<input type="text"/>
Body Site:	PERIPHERAL BLOOD		

MRN:	<input type="text"/>	Indication for Testing:	C91.00 Acute lymphoblastic leukemia not having achieved remission
Collected Date:	<input type="text"/>	Time:	<input type="text"/>
Received Date:	<input type="text"/>	Time:	<input type="text"/>
Reported Date:	<input type="text"/>	Time:	<input type="text"/>

Detected Genomic Alterations				
No evidence of mutations	No detectable autosomal chromosomal structural gain or loss	HPV viral RNA: Detected (type 82) (low level)	TTV viral RNA: Detected (27951 copies)	B and T cell clonality: Not detected

Results Summary

- **-There is no evidence of circulating neoplastic DNA/RNA in the analyzed sample.**
- **-No detectable autosomal chromosomal structural gain or loss.**
- **-B and T cell clonality: Not detected**
- **-EBV viral RNA: Not detected**
- **-HPV viral RNA: Detected (type 82) (low level)**
- **-TTV viral RNA: Detected (27951 copies)**
- **-HLA Genotyping:**
 - **-HLA-A: A*02:01-A*29:02**
 - **-HLA-B: B*07:02-B*44:03**
 - **-HLA-C: C*16:01-C*07:02**

-The lack of somatic mutations suggests absence of a neoplastic clone in the analyzed sample.

-TTV (Torque teno virus) is considered as a marker of immunocompetence in patients with immunological impairment and inflammatory disorders. TTV is regarded as a part of the human virome and, in general, does not cause pathology in immune-competent individuals. However, high TTV load is associated with increased risk of infection. In patients with organ transplant, low TTV load is associated with an increased risk of rejection.

Relevant Genes with NO Alteration

No evidence of mutation in: NOTCH, SF3B1, TP53, MYD88

Test Description:

This is a comprehensive molecular profile which uses next generation sequencing (NGS) to identify molecular abnormalities, including single nucleotide variants (SNVs), insertions/deletions (indels), copy number variants (CNVs), fusions, B- and T-cell clonality, and viruses (HPV, EBV, and TTV), in cell-free (cf) DNA of 284 genes and cfRNA in greater than 1600 genes associated with hematologic neoplasms, including leukemia, lymphoma, myeloma, myelodysplastic syndrome and myeloproliferative neoplasms. Whenever possible, clinical relevance and implications of detected abnormalities are described below.

Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes cfDNA for abnormalities in 284 genes and cfRNA of >1600 genes for abnormalities that are reported in various types of hematologic neoplasms. The assay also detects several viruses that are important in oncology, including EBV, HPV and TTV. TTV (torque teno virus) was first discovered in a patient with non-A-E hepatitis and is now regarded as a part of the human virome. In general, TTV does not cause pathology in immunocompetent individuals. TTV is considered as a marker of immune competence in patients with immunological impairment and inflammatory disorders. High TTV load is associated with increased risk of infection. In patients with organ transplant, low TTV load is associated with an increased risk of rejection.

Nucleic acid is isolated from peripheral blood plasma. Performance of the assay may vary depending on the quantity and quality of nucleic acid, sample preparation and sample age. Testing is performed using massive parallel sequencing of the coding DNA of the listed genes. This includes sequencing of all the exons as well as approximately 50 nucleotides at the 5' and 3' ends of each coding exon to detect splice site abnormalities. The TERT promoter region, including the hotspots at -124 and -146 bp, is also covered. Our cfDNA sequencing method has a sensitivity of 0.1% for detecting hot spot mutations, 0.5% for detecting single nucleotide variants (SNVs) and 1% for small (<60 bp) insertions/ deletions (indels). Known hot spots in specific genes such as IDH1/2, NRAS, and KRAS are reported at levels of 0.01% and higher when both cfRNA and cfDNA results are combined. Significant gene amplification and deletion (copy number variants) are also reported. Targeted RNA NGS is performed by hybrid capture and duplicates are excluded for levels measurements. The Universal Human Reference (UHR) RNA is used as control. All detected fusion transcripts are reported. While the major focus of the RNA analysis is the detection of fusion mRNA, mutations in the expressed RNA of the analyzed genes, B- and T-cell clonality, HLA class I genotyping, and Epstein-Barr virus (EBV), human papillomavirus (HPV) and torque teno virus (TTV) viral RNA are also analyzed and reported. The sensitivity of this assay in detecting fusion mRNA is between 5% and 10%. This test specifically covers translocations that lead to the expression of fusion RNA. Translocations that lead to deregulation of expression can be addressed by this test if compared to the expression proper normal control. Since the clinical relevance of the RNA expression level of most of the genes is not characterized at this time, only a few specific genes will be commented on when abnormalities are detected.

Based on our validation study, the following exonic regions of the genes listed below are not covered appropriately <100 X coverage and sequencing by NGS may not be reliable in these regions. The poor coverage is primarily due to the inherent difficulty in obtaining adequate sequencing coverage in regions with high GC content. No well-characterized hotspots are present in these regions. RAD51 NM_133487 chr15:40994004-40994124, BRCA1 NM_007300 chr17:41231351-41231416, FUBP1 NM_003902 chr1:78435609-78435699, CBLB NM_170662 chr3:105420938-105421303, TERT NM_198253 chr5:1295183-1295250, ARID1B NM_017519 chr6:157098715-157100605, CUX1 NM_001202543 chr7:101740644-101740781, KMT2C NM_170606 chr7:151891314-151891346 and 151935792-151935911, GALNT12 NM_024642 chr9:101569952-101570351, ATM NM_000051 chr11:108164040-108164204, CDK17 NM_001170464 chr12:96679880-96679926, RB1 NM_000321 chr13:48954189-48954220, SETBP1 NM_015559 chr18:42643044-42643692, KMT2B NM_014727 chr19:36208921-36209283, AR NM_000044

chrX:66764889-66766604, STAG2 NM_001042749 chrX:123200025-123200112.

The table below contains a partial list of the tested DNA genes. For a complete list, please go to:

<https://genomictestingcooperative.com/genomic-tests/liquid-trace-hematologic-malignancies/> (click the DNA tab)

The table below contains a partial list of the tested RNA genes (Fusions/Expression). For a complete list, please go to:

<https://genomictestingcooperative.com/genomic-tests/liquid-trace-hematologic-malignancies/> (click the RNA tab)

Tested genes

Genes Tested for Abnormalities in Coding Sequence												
ABL1	BCL2	CBL	CDKN2C	DICER1	FAS	IDH2	KMT2A	MEF2B	NSD1	PPM1D	SETD2	TERT
AKT1	BCL2L1	CBLB	CEBPA	DNMT3A	FBXW7	IGF1R	KMT2B	MPL	PALB2	PPP2R1A	SF3B1	TET2
AKT2	BCL6	CBLC	CHEK1	EP300	FLT3	IKZF1	KMT2C	MRE11A	PAX5	PTCH1	SMAD2	TGFBR2
AKT3	BCOR	CCND1	CHEK2	ERG	GATA1	IKZF3	KMT2D	MTOR	PBRM1	PTEN	SMAD4	TP53
ALK	BCORL1	CCND3	CIC	ETV6	GATA2	IRF4	KRAS	MUTYH	PDGFRA	PTPN11	SMARCA4	TSC1
AMER1	BCR	CD274	CREBBP	EZH2	GATA3	JAK1	MAP2K1	MYC	PDGFRB	RAD21	SMARCB1	TSC2
APC	BIRC3	CD79A	CRLF2	FAM175A	GEN1	JAK2	MAP2K2	MYD88	PHF6	RAD50	SMC1A	TSHR
ARID1A	BLM	CD79B	CSF1R	FAM46C	GNAQ	JAK3	MAP2K4	NFE2	PIK3CA	RAD51	SMO	U2AF1
ARID1B	BRAF	CDH1	CSF3R	FANCA	GNAS	KAT6A	MAP3K1	NFKBIA	PIK3R1	RB1	SOCS1	UBA1
ARID2	BRCA1	CDK12	CTNNA1	FANCC	H3F3A	KDM5C	MAP3K14	NOTCH1	PIK3R2	RHOA	SRC	WT1
ASXL1	BRCA2	CDK4	CTNNB1	FANCD2	HNF1A	KDM6A	MAPK1	NOTCH2	PIM1	RNF43	SRSF2	ZNF217
ATM	BTK	CDK6	CUX1	FANCE	HOXB13	KDR	MCL1	NOTCH3	PLCG1	RUNX1	STAG2	ZRSR2
ATRX	CALR	CDKN2A	CXCR4	FANCF	HSP90AA1	KEAP1	MDM2	NPM1	POLD1	SDHB	STAT3	
B2M	CARD11	CDKN2B	DDR2	FANCG	IDH1	KIT	MDM4	NRAS	POLE	SETBP1	STK11	

Electronic Signature

Maher Albitar, M.D.

The test (sample processing, sequencing and data generation) was performed at Genomic Testing Cooperative, LCA, Genomic Testing Cooperative, LCA, 175 Technology Drive, Suite 100, Irvine, CA 92618. Medical Director Maher Albitar, M.D. Analysis of the data was performed by Genomic Testing Cooperative, LCA, 175 Technology Drive, Suite 100, Irvine, CA 92618. Medical Director: Maher Albitar, M.D.

The test was developed and its performance characteristics have been determined by Genomic Testing Cooperative, LCA. This test has not been approved by the FDA. The FDA has determined such clearance or approval is not necessary. This laboratory is CLIA certified to perform high complexity clinical testing.