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Regd. Office : 5th Floor, Doctor House, Nr. Parimal Garden, Ahmedabad-380006 Gujarat
CIN: U85195GJ2009PLC057059

LABORATORY REPORT

Reg. No	:	**30402108624**	Reg. Date	:	20-Apr-2023 21:01
Name	:	**Mr. ANIRUDDHA CHAKRABARTI**	Collected on	:	21-Apr-2023 13:41
Sex/Age	:	**Male / 48 Years**	Report Date	:	27-Apr-2023
Ref. By	:	S MUKHERJEE	Tele. No	:	
Location	:	RANJAN MOULE @ KONNAGAR	Dispatch At	:	

CHRONIC LYMPHOCYTIC LEUKEMIA PANEL SUMMARY

FISH PANEL			
Clinical Indication		**Chronic Lymphocytic Leukemia**	**Prognosis**
1	FISH – Trisomy 12	Not detected	====
2	FISH – DEL 13q14	Not detected	====
3	FISH – 17p13(*TP53* gene)	Not detected	====
4	FISH – 11q22(*ATM* gene)	Not detected	====

Summary :

FISH analysis of 200 interphase cells using combination of FISH probes showed absence of trisomy of chromosome 12 and there is no evidence of deletion of chromosome 11 (*ATM* gene) ,chromosome 13 (*DLEU* gene) and chromosome 17 (*TP53* gene) in 100% cells studied.

Correlation with clinical and haematological findings and with molecular study is suggested.

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FLUORESCENCE IN SITU HYBRIDIZATION REPORT

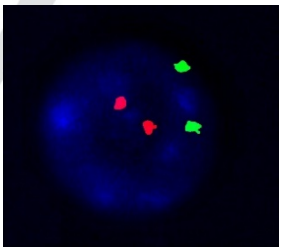
F/CG/RPT/03, Rev.:04

Sample Type : Heparinized Peripheral Blood
Clinical Diagnosis : Chronic lymphocytic leukemia
Test performed : Fluorescence In Situ Hybridization

Probe Details : XL *p53* (17p13) and *ATM* (11q22) probe: 11q22 region - SPECTRUM GREEN 17p13 region - SPECTRUM ORANGE

FISH Results *(As per cytogenetic nomenclature ISCN 2020)*

nuc ish 11q22 (*ATM* x 2), 17p13(*p53* x 2)[200/200] = 100%



Diagnostic Interpretation :

There is absence of deletion of *ATM* gene (11q22) and *TP53* gene (17p13) in 100% cells studied.

Laboratory Analysis : Interphase cells = 200
No deletion of *p53* gene and *ATM* gene = 200
deletion of *p53* gene and *ATM* gene = 000

*Cut off value for **1R 2G** signal pattern is **9%** or **18 round cells**. Hence, cells more than 9% or 18 round cells are considered as positive.

*Cut off value for **2R 1G** signal pattern is **9%** or **18 round cells**. Hence, cells more than 9% or 18 round cells are considered as positive.

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FLUORESCENCE IN SITU HYBRIDIZATION REPORT

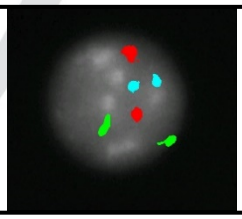
F/CG/RPT/03, Rev.:04

Sample Type : Heparinized peripheral blood
Clinical Diagnosis : Chronic lymphocytic leukemia
Test performed : Fluorescence In Situ Hybridization

Probe Details : XL DLEU/LAMP$_1$/12cen orange/green/blue DNA probe:12p11.1-12q11 region - SPECTRUM GREEN, 13q14 (DLEU) - SPECTRUM ORANGE , 13q34 (LAMP$_1$) - SPECTRUM AQUA

FISH Results (As per cytogenetic nomenclature ISCN 2020)

nuc ish 13q14(DLEU x2),13q34 (LAMP$_1$ x2), (12p11.1-12q11 x 2))[200/200] = 100%



Diagnostic Interpretation :

There is no evidence of trisomy 12 in 100% cells studied.

Laboratory Analysis : Interphase cells counted = 200

Deletion of DLEU region = 000
No deletion of DLEU / LAMP$_1$ region = 200
Trisomy of chromosome 12= 000
Normal copies of chromosome 12 = 200

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FLUORESCENCE IN SITU HYBRIDIZATION REPORT

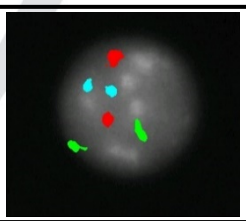
F/CG/RPT/03, Rev.:04

Sample Type : Heparinized peripheral blood
Clinical Diagnosis : Chronic lymphocytic leukemia
Test performed : Fluorescence In Situ Hybridization

Probe Details : XL DLEU/LAMP$_1$/12cen orange/green/blue DNA probe:12p11.1-12q11 region - SPECTRUM GREEN, 13q14 (DLEU) - SPECTRUM ORANGE , 13q34 (LAMP$_1$) - SPECTRUM AQUA

FISH Results (As per cytogenetic nomenclature ISCN 2020)

nuc ish 13q14(DLEU x2),13q34 (LAMP$_1$ x2), (12p11.1-12q11 x 2))[200/200] = 100%



Diagnostic Interpretation :

There is no evidence of deletion of DLEU region of chromosome 13 in 100% cells studied.

Laboratory Analysis : Interphase cells counted = 200

Deletion of DLEU region = 000	Trisomy of chromosome 12= 000
No deletion of DLEU / LAMP$_1$ region = 200	Normal copies of chromosome 12 = 200

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MOLECULAR ANALYSIS of *IGH* Somatic Hypermutation Assay

Nucleic Acid Type used: Peripheral Blood (EDTA)

Method of Analysis: DNA was extracted using 200µl of EDTA blood by using QIAamp gDNA mini kit as per manufacturer's instructions. The assay was performed using *IGH* Somatic Hypermutation Assay v2.0. The test employs two different master mixes: Hypermutation Mix 1 v2.0 and Hypermutation Mix 2 v2.0. The Hypermutation Mix 1 v2.0 targets sequences between the leader and joining regions. Therefore the amplicon product(s) span the entire variable (V) region, which contains the FR1, CDR1 (complementarity-determining region 1), FR2, CDR2, FR3 and CDR3 regions. The Hypermutation Mix 2 v2.0 assay targets sequences between the framework 1 (FR1) and joining (J) regions. The amplicons were denatured and subjected to fragment analysis using gene mapper software on the 3500Dx genetic analyzer (Applied bio systems, USA). Amplicons are than subjected to sanger sequencing using sequencing primers supplied with the kit. Sequences were analyzed using IMGT/V-QUEST tools followed by Stereotypic subset identification.

Stereotype Analysis method: Stereotype Analysis was done using ARResT/AssignSubsets tool. This analysis is for Research Use Only.

Results:

Result summary: 8624_ANIRUDDHA_IGVH_MIX1_HYPERMUTATION	Productive IGH rearranged sequence (no stop codon and in-frame junction)		
V-GENE and allele	Homsap IGHV4-34*02 F	score = 1281	identity = 94.39% (269/285 nt)
J-GENE and allele	Homsap IGHJ4*02 F	score = 131	identity = 96.43% (27/28 nt)
D-GENE and allele by IMGT/JunctionAnalysis	Homsap IGHD5-18*01 F	D-REGION is in reading frame 3	
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[25.17.38.4]	[8.7.14]	CARSLGHTYDYYFDHW
JUNCTION length (in nt) and decryption	48 nt = (9)-2{6}-2(13)-5{2}+1(17)	(3'V)3'{N1}5'(D)3'{N2}5'(5'J)	

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Stereotype Analysis

SeqCure ID [index]	level	stage	IMGT/V-QUEST basic info / ARResT/SeqCure message
[#0]			341nt ; CARSLGHTYDYYFDHW ; IGHV4-34*02 F ; 94.39% ; IGHD5-18*01 F ; IGHJ4*02 F ; searched for indels:nounkround 1productive
		round 1	productive

Assignment report table

Label [+ heat map, if appl.]	SeqCure	Subset	Confidence	Score
ANIRUDDHA	OK	unassigned	extreme	-Inf

Interpretation

Sample Status: Mutated (Identity 94.39%)

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Subset: unassigned

BACKGROUND INFORMATION:

Rearrangements of the antigen receptor genes occur during ontogeny in B and T lymphocytes. These gene rearrangements are unique in length and sequence for each cell. Therefore, polymerase chain reaction (PCR) assays can be used to identify lymphocyte populations derived from a single cell by detecting the unique V-J gene rearrangements present within these antigen receptor loci. This PCR-based assay employs multiple consensus DNA primers that target conserved genetic regions within the immunoglobulin heavy chain (*IGH*) gene. This test is used to detect and sequence the majority of clonal *IGH* rearrangements from either genomic DNA or complementary DNA (cDNA).Clonal products are detected using capillary electrophoresis and sequenced using provided sequencing primers by bi-directional sequencing. Hypermutation of the *IGHV* gene is strongly predictive of a good prognosis while lack of mutation predicts a poor prognosis. It should be emphasized that the results of any molecular test should always be interpreted in the context of clinical, histological and immunophenotypic data.

Limitation of Assay:

- This assay is performed using RUO kit.
- This assay does not identify 100% of clonal cell populations.
- This assay cannot reliably detect less than 5 positive cells per 100 normal cells.

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- The results of molecular clonality tests should always be interpreted in the context of clinical, histological and immunophenotypic data.
- PCR-based assays are subject to interference by degradation of DNA or to inhibition of PCR due to EDTA, heparin and other agents.

References:

- Miller, JE, Wilson, SS, Jaye, DJ, Kronenberg, M. An automated semiquantiative B and T cell clonality assay. Mol. Diag. 1999, 4(2):101-117.
- Instructions for Use IGH Somatic Hypermutation Assay v2.0

---------- End Of Report ----------

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