Overview of ERIC recommendations for *TP53* analysis – 2024 Update

ERIC recommendation			Notes and alternatives
Patients	Sampling	Always when deciding about treatment in both the frontline and the relapsed/refractory setting.	
Material	Type of material	Peripheral blood (PB)	Bone marrow, lymph nodes – suitable alternatives if PB lymphocyte count is low, e.g. in SLL/CLL, relapse in lymph nodes. Fresh/frozen tissues are strongly preferred.
	Tumor cell enrichment	Optimally separate $CD19^+$ cells. Alternatively, choose the method of separation based on content of CLL cells, if the information about the blood count is available.	Separation of mononuclear cells is sufficient for most cases at treatment initiation. The separation of $CD19^+$ lymphocytes is necessary when the proportion of CLL cells in the sample is low ($ALC \leq 10x10^9/l$)
	Nucleic acid	DNA	RNA analysis carries a risk of omitting truncating variants.
	Covered region	Optimum: exons 2-11 (coding region), Minimum: exons 4-10, Always include splice sites (at least +/-2 intronic bp)	
Procedure – **Sanger sequencing**	PCR protocol	Check primer sequences for presence of population variants.	
	Sequencing	Both strands (forward + reverse)	
	Data analysis	Use software designed for somatic variant detection	Free web-based software GLASS[61] is accessible via ERIC website.
Procedure – **NGS – preferred methodology**	Library preparation	Amplicon or capture-based approaches are applicable. DNA input should be sufficient to achieve the aimed limit of detection.	Several ready-to use kits involving *TP53* analysis are commercially available.
	Limit of detection (LoD)	Should be set to detect low-VAF variants (≤5% VAF).	Either variant-specific LoD or general LoD ensuring calling of >99% of all variants.
	Sequencing depth	Covering all bases in the coding region with a sufficient number of reads should be a standard.	≥99% minimum coverage percentage should be reported.
	Data analysis	Pipeline set to reliably distinguish variants from background noise	Commercial or in-house bioinformatics pipelines are applicable.
	Validation	Validate/verify the method before introducing it into diagnostics	Continuous monitoring of quality and external quality assessment is necessary.
Interpretation and reporting	Variant description	Use HGVS nomenclature: http://varnomen.hgvs.org/[83] Report the cDNA and protein level including reference sequence.	
	Interpretation	Check the variant functionality in locus-specific databases: The TP53 database: https://tp53.isb-cgc.org/[98] TP53 web site: http://p53.fr/[99] with embedded tool Seshat[85]	Interpretation algorithm provided as a part of these recommendations
	Populational and benign variants	It is preferred not to include (likely) benign variants in the report.	Check the variants with preserved functionality using gnomAD[62] and The ClinGen Evidence Repository of curated variants[88].
	VAF cut-off for reporting	Report all variants above the validated limit of detection.	The laboratory is responsible for issuing the correct result, clinical decision-making is within the responsibilities of the referring clinician
	Report form	Should follow ISO15189 Medical laboratories — Requirements for quality and competence[68].	A template report form is available on the ERIC website.

PB – peripheral blood, BM – bone marrow, ALC – absolute lymphocyte count, NGS – Next generation sequencing, VAF – Variant allele fraction