Participant G12156

External Quality Assessment (EQA)
Individual Laboratory Report

Version: Final

Member of UK NEQAS consortium

2025 Chronic Lymphocytic Leukaemia (CLL) TP53 mutation analysis

Mean Overall Score	
Genotype	2.00
Interpretation	Not marked
Clerical accuracy	2.00

Conclusions	
Recommendations	None
Performance	Satisfactory

Case 1

Category	Score	Comments
Genotype	2.00	Correct result.
Interpretation	--	Interpretation not marked. No clinical interpretation provided in the report.
Clerical accuracy	2.00	No clerical errors.

Case 2

Category	Score	Comments
Genotype	2.00	Correct result.
Interpretation	--	Interpretation not marked. No clinical interpretation provided in the report.
Clerical accuracy	2.00	No clerical errors.

Case 3

Category	Score	Comments
Genotype	2.00	Correct result.
Interpretation	--	Interpretation not marked. No clinical interpretation provided in the report.
Clerical accuracy	2.00	No clerical errors.

Thank you for your participation

Notes:

- To be read in conjunction with the EQA Summary Report
- The maximum score for each category is 2.00
- There are only two categories of performance for any EQA; 'Satisfactory' and 'Poor'

info@genqa.org • www.genqa.org • +44 (0)131 242 6898

Edinburgh Office

Laboratory Medicine, NHS Lothian
NINE, Edinburgh BioQuarter
Little France Road
Edinburgh EH16 4UX
United Kingdom

Oxford Office

Women's Centre, John Radcliffe Hospital
Oxford University Hospitals NHS Foundation Trust
Headley Way
Oxford OX3 9DU
United Kingdom

Chronic Lymphocytic Leukaemia (CLL)*TP53* 2024 EQA Summary Report

Post-Appeals: Final

GenQA Genomics Quality Assessment

UK NEQAS Leucocyte Immunophenotyping

Members of the UK NEQAS *consortium*

Introduction

Thank you for participating in the External Quality Assessment/Proficiency testing (EQA/PT) for *TP53* mutation analysis in chronic lymphocytic leukaemia (CLL) 2024. This EQA/PT is provided as a collaboration between two UK NEQAS centres: Genomics Quality Assessment (GenQA) and UK NEQAS for Leucocyte Immunophenotyping (LI), in association with ERIC (European Research Initiative on CLL).

Your individual laboratory report (ILR) is available on the GenQA website (www.genqa.org). If you have any problems accessing your scores then please contact the Scheme on info@genqa.org.

The purpose of this EQA is to assess:

- The accuracy of the analytical result;
- The interpretation of the results;
- The correct use of international nomenclature[1,2];
- The format of the report against ISO15189[3].

As part of this EQA, participants were also requested to complete an additional survey regarding methods and detailed results of testing. The summary of the EQA participation, samples provided, marking criteria applied and the scoring is provided in Appendix 1, with the results of the survey in Appendix 2.

EQA Summary Outcomes

Case 1

Name[a]	Date of Birth[b]	Gender	Sample Type
Hans MULLER	14/01/1953	Male	DNA (extracted from blood)
Referral reason			
CLL now requiring treatment			
Validated results			
Variant *Ref Seq:* NM_000546.6	**Median Variant Allele Frequency (VAF)**	**Classification**	
No clinically significant variant detected	NA	NA	

[a]Surname is in capital letters, [b]date of birth given as dd/mm/yyyy, NA: Not applicable

In general, it was observed that:

- All participants (72) submitted a correct result for this case;

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GenQA Oxford Office
Women's Centre, John Radcliffe Hospital
Oxford University Hospitals NHS Foundation Trust
Headley Way
Oxford, OX3 9DU, UK.

GenQA Edinburgh Office
Laboratory Medicine, NHS Lothian
NINE, Edinburgh BioQuarter
Little France Road
Edinburgh, EH16 4UX, UK

UK NEQAS LI Office
4th Floor Pegasus House
463A Glossop Road
Sheffield, S10 2QD, UK
Tel: +44 (0) 114 2673600

- The validated results did not detect any clinically relevant variant in *TP53;*
- This sample was sent specifically to determine whether there was a clinically significant variant of *TP53* prior to treatment. It is therefore important to specifically state in the summary statement that no clinically significant/pathogenic variant was detected;
- The majority of laboratories stated this information. Four laboratories (4/72, 5%), all of whom performed panel testing and reported the presence of variants in other genes in the summary statement, did not state the specific result for *TP53*;
- The majority of laboratories included an interpretation of the results (67/72, 93%). Five laboratories submitted a genotype only report that did not contain an interpretation section (4/5) or only provided a generic interpretation provided by the software (1/5);
- There were no critical interpretation errors for this case.

Case 2

Name[a]	Date of Birth[b]	Gender	Sample Type
Adam TING	15/07/1950	Male	DNA (extracted from blood)
Referral reason			
CLL now requiring treatment			
Validated results			
Gene Reference NM_000546.6	**Median Variant Allele Frequency (VAF)[c]**	**Classification[d]**	
c.456_465del p.(Pro153AlafsTer14) c.471_487del p.(Arg158GlnfsTer17)	48.7% 48.6%	Pathogenic, Class 5[e]	

[a]Surname is in capital letters, [b]date of birth given as dd/mm/yyyy, [c] Median VAFs reported by participants, [d]Classification according to ACMG (2015)[4] (Li *et al*., 2017[5] and alternative classification systems accepted, where appropriate),[e] Likely pathogenic, Class 4 also accepted.

In general, it was observed that:

- The majority of participating laboratories (70/72, 97%) submitted a correct result for this case;
- The validated results showed the presence of two TP53 variants:
  - c.456_465del p.(Pro153AlafsTer14) variant with a VAF of 48.7%;
  - c.471_487del p.(Arg158GlnfsTer17) variant with a VAF of 48.6%.
- Manual inspection in IGV showed the cumulative presence of these variants in nearly 100% of reads;
- In addition, sequencing data showed that they are located on different alleles (*trans*). Nine laboratories correctly stated this in their reports. It was not expected that this would be reported;
- Both variants were reported by the majority of laboratories (70/72, 97%):
  - All participants correctly identified the c.456_465del p.(Pro153AlafsTer14) variant;
  - However, two laboratories did not identify the c.471_487del p.(Arg158GlnfsTer17) variant and received a critical genotyping error.
- These variants should be classified as Pathogenic, but Likely pathogenic was also accepted;
- In total, 71 laboratories (71/72, 98.6%) provided the pathogenicity on their reports. One laboratory stated that they don't routinely provide this information on their report;
- Standard terminology, such as strongly clinically significant, Tier 1, pathogenic etc, was used by 69 participants (69/71, 97%) who classified the variants [4-7]:
  - Two participants described the variants as mutated.
- All laboratories using next generation sequencing (NGS) included the VAF of the variants on their report;

- A clinical interpretation was provided by 67 laboratories (67/72, 93%). Five laboratories submitted a genotype only report that did not contain an interpretation section (4/5) or only provided a generic interpretation provided by the software that was not sufficient and was therefore not marked (1/5);
- There were no critical interpretation errors for this case.

Case 3

Name[a]	Date of Birth[b]	Gender	Sample Type
Ann WOOD	29/05/1947	Female	DNA (extracted from blood)
Referral reason			
CLL now requiring treatment			
Validated results			
Variant *Ref Seq:* NM_000546.6	**Median Variant Allele Frequency (VAF)[c]**	**Classification[d]**	
c.827C>A p.(Ala276Asp)	33%	Pathogenic, Class 5[e]	

[a]Surname is in capital letters, [b]date of birth given as dd/mm/yyyy, [c] Median VAFs reported by participants [d]Classification according to ACMG (2015)[4] (Li *et al*., 2017[5] and alternative classification systems accepted, where appropriate),[e] Likely pathogenic also accepted.

In general, it was observed that:

- All participants (72) submitted a correct result for this case;
- The validated results showed the presence of a c.827C>A p.(Ala276Asp) variant with a VAF of 33%;
- All participants (72/72, 100%) correctly identified the variant in this case;
- These variants should be classified as pathogenic, but likely pathogenic was also accepted;
- In total, 71 (71/72, 98.6%) provided the pathogenicity on their reports. One laboratory does not routinely provide this information on their report;
- Standard terminology, such as strongly clinically significant, Tier 1, pathogenic etc, was used by 69 participants (69/71, 97%) who classified the variants [4-7]:
  - Two participants described the variants as mutated.
- All laboratories using NGS included the VAF of the variant on their report;
- A clinical interpretation was provided by 67 laboratories (67/72, 93%). Five laboratories submitted a genotype only report that did not contain an interpretation section (4/5) or only provided a generic interpretation provided by the software that was not sufficient and was therefore not marked (1/5);
- There were no critical interpretation errors.

All cases

General Findings

- Overall, all cases were very well reported;
- There were only two critical genotyping errors in case 2 where one of the two variants present was not detected;
- Analysis of the *TP53* gene in patients with CLL should include exons 4-10 as a minimum, according to NM_000546.6, and a description of the genomic regions analysed should be provided in the report[8]:
  - Two laboratories did not state which exons were analysed in the report;
  - One laboratory did not analyse exons 4-10 and received a deduction;

- One laboratory provided discordant information about whether exons 4-10 were analysed and received a deduction.

- Reports should clearly state both the gene reference **number** and **version** used[8]:
  - 65 participants included this information on their report of which 27 (27/65, 41%) provided no, or not the current, version number;
  - Four participants provided the Ensembl transcript number instead and one provided the LRG reference;
  - Two provided no reference sequence nor other reference and received a deduction.
- The pathogenicity of the variant should be included in the report. For variant classification it is recognised that there are two main references[4,5] and terminology from either source is recommended (terminology from Horak *et al*.[6] also accepted). Non-standard terminology, such as mutated, prognostic or actionable, should be avoided in clinical reports. In addition, it is good practice to include the reference used for classification on the report;
- A description of the clinical consequence of the variant should be provided. It is recommended to include a brief conclusion summarizing the possible prognostic impact or resistance in the report along with a reference to the corresponding literature. However, it is recognised that differences exist between countries regarding the responsibility of the laboratory and the clinician and that the content of this conclusion may vary;
- It is essential that the report includes a statement that NGS and Sanger sequencing cannot distinguish between somatic and germline variants without follow-up testing of normal tissue from the same individual[9]. It is recommended to include this within the details or information about the test;
- A VAF of around 50% may suggest a germline origin in some circumstances. However, care must be taken not to overemphasise a possible germline origin in CLL. This is a very rare occurrence in CLL and therefore overinterpretation should be avoided;
- Compared to previous years, there were fewer recommendations for genetic counselling in the reports. For case 2, with a VAF around 50%, only two laboratories suggested genetic counselling. There were no recommendations in the reports for case 3;
- It is important to provide sufficient technical/analytical information, as well as the limitations of the test, in the report. There was great variation in the detail provided by participants. As a minimum, reports should include:
  - A description of the test methodology, including the exons analysed, the commercial kit name and version number or in-house method used (e.g., amplicon based);
  - The limitations of the test were expected to include the limit of detection for a variant;
    - Two laboratories did not include the limit of detection of the technique on their report and received a deduction.
  - Also, it may be beneficial to highlight where sequencing platforms cannot detect large duplications and deletions within the tested regions of *TP53*;
  - As, outlined above, a statement concerning the distinction of germline/somatic variants should be included. The majority of participants who submitted a full clinical report included such a statement, at least for cases 2 and 3, although 16/72 (22%) omitted this on all reports. These participants received a comment without deduction.
- Seven participants reported the presence of variants in other genes in their reports. Variants should only be reported in genes which are requested within the Scheme and therefore have validated results associated with them;
- Care should be taken when reusing template reports, from other EQA cases or from previous EQA submissions, to ensure that all fields in the reports (patient/sample details, results, etc) are updated correctly as these incurred deductions for some submissions.

Nomenclature

- Variants should be described according to HGVS nomenclature[1]. Both the long and short versions of the nomenclature were accepted. The HGVS nomenclature standard was high for this EQA, only four participants used incorrect nomenclature for at least one of the cases.

Clerical accuracy

- This EQA required the submission of a complete clinical report for each case that complies with European reporting guidelines[8], ERIC guidelines[10] and ISO15189[3] requirements. The overall standard of reporting was good with few omissions, appropriate inclusion of the clinical relevance of the result and a high adherence to the report format recommended by ERIC (http://www.ericll.org/guidance-toolstp53/);
- There was a subset of recurrent omissions which meant the reports did not comply with ISO 15189[3] as follows:
  - Five participants (5/72, 7%) did not provide pagination on the report;
  - Four participants did not anonymise their reports (4/72, 5%);
  - Three participants (3/72, 4%) did not include a referral reason on their reports;
  - Seven participants (7/72, 9.7%) did not include patient identifiers on each page of the report;

  There were no deductions for these omissions.

Appeals

There were no appeals for this EQA.

References

1. Human Genome Variation Society (HGVS) nomenclature HGVS Sequence Variant Nomenclature. http://varnomen.hgvs.org/
2. den Dunnen JT., *et al.* HGVS Recommendations for the Description of Sequence Variants: 2016 Update. Human Mutation. 2016;37:564-569
3. ISO 15189:2022. Medical laboratories – requirements for quality and competence.
4. Richards S., *et al.* (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med;17:405–424.
5. Li MM., *et al.* (2017). Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn;19:4-23.
6. Horak P., *et al.* (2022). Standards for the classification of pathogenicity of somatic variants in cancer (oncogenicity): Joint recommendations of Clinical Genome Resource (ClinGen), Cancer Genomics Consortium (CGC), and Variant Interpretation for Cancer Consortium (VICC) Genet Med;24:986-998.
7. Fortuno C., *et al.* Specifications of the ACMG/AMP variant interpretation guidelines for germline TP53 variants. *Hum Mutat* 2021 Mar; 42(3): 223-236.
8. Deans Z., *et al.* (2022). Recommendations for reporting results of diagnostic genomic testing. Eur. J. Hum. Genet. https://www.doi.org/10.1038/s41431-022-01091-0.
9. Leroy B., *et al.* (2017). Recommended guidelines for validation, quality control, and reporting of TP53 variants in clinical practice. Cancer Res;77:1250–60.
10. Malcikova J., *et al.* (2024) ERIC recommendations for TP53 mutation analysis in chronic lymphocytic leukemia-2024 update. Leukemia 38(7):1455-1468.
11. GenQA Haematological Neoplasms EQA Performance criteria https://genqa.org/performance-monitoring.

Assessors

GenQA and UK NEQAS LI would like to thank the specialist group of assessors who undertook the analysis of the returns.

Assessor	Location
Panagiotis Baliakas	Uppsala, Sweden
Mark Catherwood	Belfast, Northern Ireland, UK
Jack Cowland	Copenhagen, Denmark
Sarka Pavlova	Brno, Czech Republic
Ilaria Scarpelli	Bern, Switzerland

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Future EQAs

If you have any comments on how this EQA might be improved in the future or you require any further information, please email info@genqa.org

Thank you for participating in this EQA. We hope you found participation useful and will continue to participate in future.

Registration for 2025 EQAs is now open.

Prof Sandi Deans
GenQA Director

Stuart Scott
UK NEQAS LI Director

Amendments to the Summary EQA report following the appeals process
The EQA report was issued as a Pre-appeals version 1 on 3rd March 2025.

Section	Amendment
Title	**Added:** *Post-appeals Final*
Appeals	**Deleted:** The scores are subject to appeal. If you wish to appeal against any deduction then please do so by **Friday 21st March 2025**, 23:59h GMT. Participants can submit an appeal by using the *Appeals submission form* available from the EQA webpage. All appeals will be considered anonymously by the Cancer Genomics Specialist Advisory Group joint workgroup and the outcome of your appeal will be available via your website account. You will be notified by email when the appeals outcome and the final scores are published, and the EQA Summary Report will be amended if required and released as the Final version. **Replaced with:** There were no appeals for this EQA.

This report is the EQA Summary Report Final version 2 and supersedes the Pre-appeals version 1.

Authorisation/Approval

This document has been authorised/approved on behalf of GenQA by: Sandi Deans on 7th April 2025.

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Appendix 1: Additional information

Samples provided and testing required

Three DNA samples were distributed, referral information and clinical details were provided online. Participants were expected to report their findings in the context of the clinical case scenario.

Validated results

All cases were validated independently by two laboratories prior to distribution without knowledge of the expected result.

Marking criteria

The following peer reviewed marking criteria was used (*Table 1*). Where the information was missing but required by Best Practice, marks were deducted. If a critical analytical error is given, the interpretation and clerical accuracy are not marked.

Table 1: Summary of marking criteria

Case	Category	Criteria	Marks awarded
1	Genotyping	No clinically significant *TP53* variant detected.	2.0
	Interpretation	No clinically significant *TP53* variant detected.	2.0
2	Genotyping	c.456_465del p.(Pro153AlafsTer14) *TP53* variant detected. c.471_487del p.(Arg158GlnfsTer17) *TP53* variant detected.	2.0
	Interpretation	A Pathogenic/Likely pathogenic *TP53* variant was detected. *TP53* mutations are associated with adverse prognosis and poor response to chemo(immuno)therapy.	2.0
3	Genotyping	c.827C>A p.(Ala276Asp) *TP53* variant detected.	2.0
	Interpretation	A Pathogenic/Likely pathogenic *TP53* variant was detected. *TP53* mutations are associated with adverse prognosis and poor response to chemo(immuno)therapy.	2.0

Other minor deductions

Genotype deduction of 0.5 marks:

- Testing should cover at least exons 4 to 10 of the *TP53* gene according to NM_000546.6
- Incorrect use of HGVS nomenclature

Interpretation deduction of 0.5 marks:

- An appropriate reference sequence not included in the report
- Reporting limitations of detection not included in the report
- The methodology used not included in the report
- The clinical consequence of the variant and summary of the finding in the context of the current knowledge not included in the report

Participation and scoring submissions

Seventy-two laboratories registered to participate in this EQA and all submitted an EQA return (100%) *(Table 2)*.

Only two categories of performance in any single EQA are defined: **satisfactory** and **poor**[11]. Satisfactory performance is defined as the standard that should be achievable by all participants.

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Of the 72 laboratory submissions that were marked, two received a critical genotyping error *(Table 3)*. Participants are encouraged to refer to the histogram (*Figure 1 and Table 4*) summarising the performance of all the participants and decide through their own benchmarking whether they consider their performance to be acceptable. Each category (genotype, interpretation and clerical accuracy) is marked out of 2.

Table 2: Summary of EQA results

Status	Number of participants
Enrolled	72
Participated	72
Satisfactory performance	70 (97%)

Table 3: Summary of critical errors per EQA case

Case	Number of Critical Errors		Total number participants participating
	Genotyping[f]	Interpretation	
1	0	0	72
2	2	0	72
3	0	0	72

[f]The interpretation and clerical accuracy were not marked if there was a critical genotyping error.

Figure 1: Distribution of overall average scores for genotype, interpretation and clerical accuracy for all submitting participants

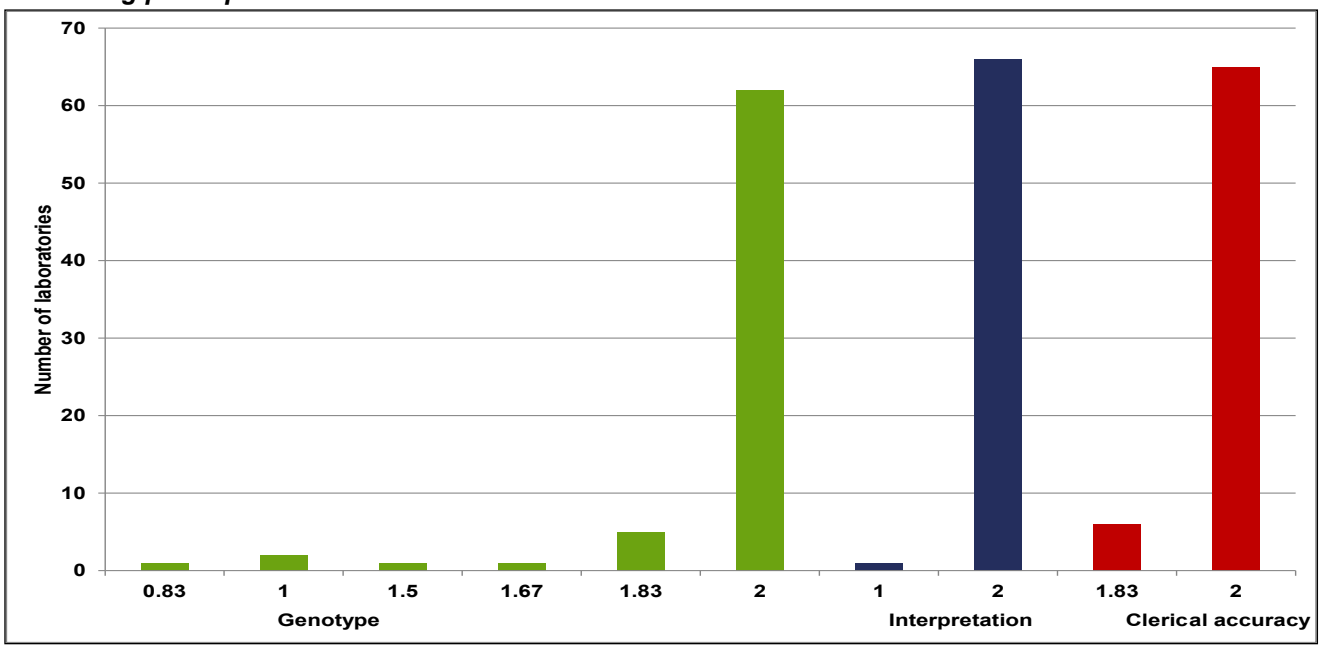

Table 4: Mean scores of all participating participants (maximum score = 2)

Category	Case 1[g]	Case 2	Case 3
Mean Genotype Score	1.97	1.88	1.94
Mean Interpretation Score	1.99	1.98	1.99
Mean Clerical Accuracy Score	1.96	2.00	2.00

[g]As some deductions are only applied once to case 1, the mean scores for case 1 may be lower than for other cases.

Scheme compliance

Four participants did not anonymise their reports. In order to ensure the impartiality of the marking process, please ensure all submitted documents are anonymised. This includes laboratory logos, staff names and accreditation numbers.

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Appendix 2: Survey of laboratory practice

As part of this EQA, participants were requested to complete an additional survey regarding methods and detailed results of testing.

Fifty-six of 72 participants registered in the programme provided method data in the additional survey. Please note figures in the tables below may not tally with the total number of participants returning results, nor to numbers elsewhere in the report, due to some participants not returning all data requested or using multiple techniques.

Table 5: Sequencing method used

	Participants
Next generation sequencing (NGS)	48
Sanger sequencing	6
Both Sanger and NGS	1
Nanopore sequencing	1

Table 6: NGS platform used (to analyse the sample(s) in this trial)

	Participants
Illumina NextSeq	12
Illumina MiSeq	12
Illumina NovaSeq	9
Life Tech Ion S5	9
Life Tech Ion S5 XL	3
Life Tech Genexus	2
Illumina MiniSeq	1
MGI DNBSEQ-G400	1
ONT MinION	1

Table 7: NGS protocol

	Participants
Commercial kit for NGS library preparation	33
In-house primers and protocol	10
Custom TWIST panel	4
TP53 community panel	2
Custom Qiaseq Panel	1
Custom QIAseq targeted DNA panel (QIAGEN)	1
Ampliseq Liverpool Lymphoma Network Panel	1

Table 8: Sanger sequencing protocol

	Participants
In-house primers and protocol	6
IARC *TP53* primers and protocol	2
IARC *TP53* primers with modified protocol	2
Other	1

Table 9: Annotation database resources routinely used during variant interpretation process

	Participants
The TP53 Database (originally WHO International Agency for Research on Cancer (IARC) TP53 Database) https://tp53.cancer.gov/	48
ClinVar (NCBI)	45
COSMIC (Catalogue Of Somatic Mutations In Cancer)	45
TP53 website (UMD database http://p53.fr/)	35
The Genome Aggregation Database (gnomAD)	28
dbSNP (Short Genetic Variations, NCBI)	21
Varsome (Aggregation tool)	20
Seshat	20
Alamut	18
FRANKLIN	16
In-house	10
My Cancer Genome (Vanderbilt-Ingram Cancer Center)	10
OMIM (NCBI)	7
HGMD (The Human Gene Mutation Database)	7
https://mutantp53.broadinstitute.org/	5
The Cancer Genome Atlas (TCGA)	4
PHANTM	3
Qiagen QCI Interpret	2
Cancer Hotspots	1
Molecular Tumor Board Portal (MTBP)	1
OncoKB	1
cBioportal	1
Variant Validator	1
QIAGEN Clinical Insights Interpret One software	1

Figure 2: Histogram depicting exons routinely analysed by participants (in this clinical context)

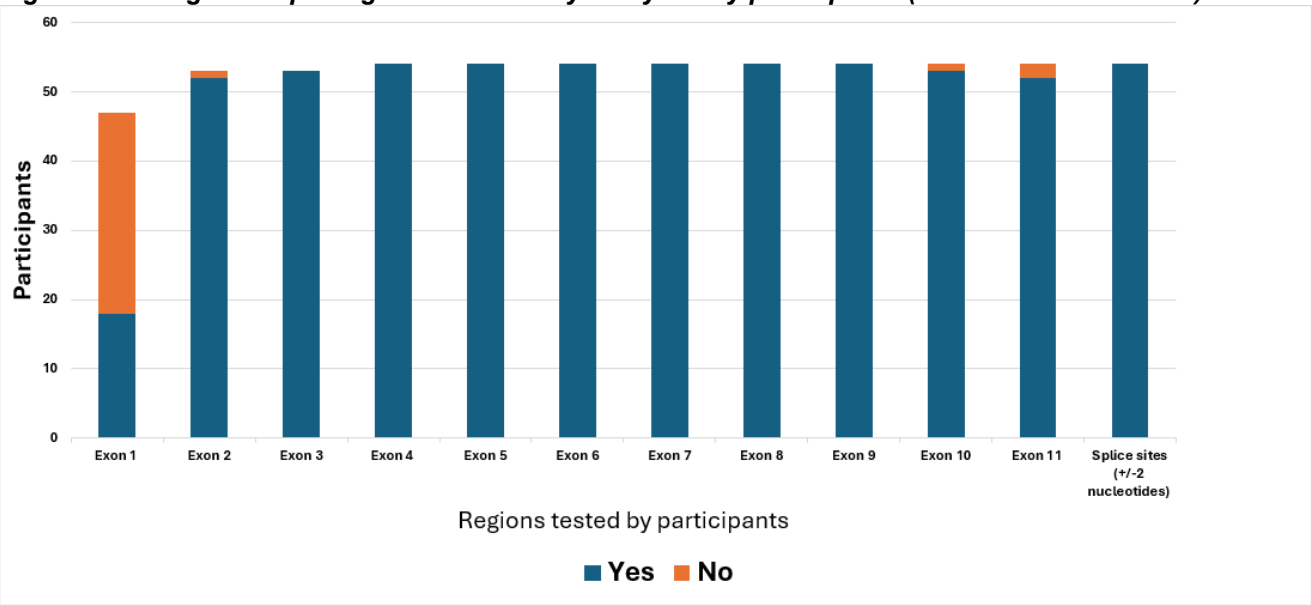

Table 10: Genome Assembly

	Participants
GRCh37/hg19	41
GRCh38	17

Table 11: Minimum variant allele frequency (VAF) for reporting identification of a variant

	Participants
0.5%	1
1%	6
2%	4
2.5%	1
3%	8
5%	29
10%	3
15%	2
20%	2

Methodology

- In this round, 48 (85.7%) participants used NGS, six (10.7%) participants used Sanger sequencing, one participant (1.8%) used both NGS and Sanger sequencing and one participant (1.8%) used Nanopore sequencing. The preferred method for *TP53* variant detection in CLL is NGS; however, Sanger can be used if NGS is not available[1].

- Of those participants who returned exon sequencing information, all participants sequenced exons 3-9 and splice sites, 38.2% sequenced exon 1, 98.1% sequenced exon 2 and 10, and 96.3% sequenced exon 11. Please refer to histogram 2. The low coverage of exon 1 is likely due to the fact this exon is untranslated. The optimum regions for testing recommended by the 2024 ERIC *TP53* recommendations are exons 2-11 (coding region) with a minimum of exons 4–10, always including splice sites (at least ±2 intronic bp)[1].

Annotation and interpretation

- The TP53 Database (formerly the WHO International Agency for Research on Cancer (IARC) TP53 database (n = 48), COSMIC (n = 45), ClinVar (NCBI) (n = 45), TP53 website (UMD database http://p53.fr/) (n=35), The Genome Aggregation Database (gnomAD) (n=28), dbSNP (Short Genetic Variations, NCBI) (n=21), Varsome (Aggregation tool) (n=20) and Alamut (n=18) remain the annotation resources most widely utilised by participants. A list of databases important to the interpretation of TP53 variants can be found in supplementary Table S5 of the 2024 ERIC TP53 recommendations[1].
- Variant databases and aggregation tools should be used with caution. Submissions may not be subject to a level of curation sufficient for clinical diagnostic application; it is prudent to check the underpinning publication and/or supporting source information. Many resources access the same primary dataset(s); laboratories are encouraged to be mindful of duplicated evidence when classifying variants in terms of clinical significance.
- The most common minimum variant allele frequency (VAF) for reporting applied by participants was 5% (n = 29, 51.8% of responses, range 0.5% - 20%). The clinical significance of detecting low-level *TP53* mutations remains controversial; however, the 2024 ERIC recommendations propose a limit of detection (LOD) of ≤ 5% VAF, with laboratories to report all variants above their established LOD. Laboratories must locally determine and document their specific detection limits and analytical performance characteristics in their reports[1].