UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Pilot Lymphoid Gene Panels (Not Accredited)

Distribution - Lymphoid GP 222301

Participant –

Date Issued – 07 September 2022

Closing Date – 21 October 2022

Trial comments

This trial was issued to 43 participants, of which 37 (86.0%) returned results. Three participants informed us of their intended non return of results.

Please note this programme was previously titled Chronic Lymphocytic Leukaemia (CLL) Gene Panels (Pilot – Not Accredited). This programme has expanded to encompass a broader range of lymphoid malignancies. To facilitate a quicker turnaround time for trial report publication, one Lymphoid Gene Panels (Pilot – Not Accredited) distribution will focus on summarising the variants detected by participants (including methodological aspects) and the other will additionally provide educational elements related to variant biological classification and clinical interpretation.

Sample comments

One lyophilised sample (Lymphoid GP 105) was prepared and distributed by UK NEQAS LI. Sample Lymphoid GP 105 was manufactured using cell line material and should be considered to be from a patient with a working diagnosis of CLL.

Sample Lymphoid GP 105

Did you detect a reportable DNA sequence change in Sample Lymphoid GP 105: Yes

Your variant results

Gene	Your DNA sequence variant detected	Your protein variant	Your variant classification
TP53	c.949dup	p.Gln317Argfs*20	Strong
CCND3	c.860T>A	p.Val287Asp	Potential

All participant results

Please note, in the interests of clarity we will only summarise variants reported by ≥2 participants.

Gene	n#	Variant classification^			Variant detected (consensus)*		Median VAF (%) (range)+
		Strong clinical significance	Potential clinical significance	Unknown clinical significance	DNA sequence description	Protein level description	
TP53	35/37	28	5	1	c.949dup	p.(Gln317Prosfs*20)¥	97.3 (28.7-100.0)
CARD11	12/18	0	3	9	c.1222C>T	p.(Arg408Cys)	38.2 (32.0-43.0)
CCND3	4/5	0	2	2	c.860T>A	p.(Val287Asp)	51.0 (50.0-56.6)
PTPRD	3/5	0	1	2	c.2321A>T	p.(Gln774Leu)	66.0 (64.7-68.0)
RPS15	2/8	0	0	2	c.217C>T	p.(Pro73Ser)	- (10.0-21.0)

#Total number of participants reporting this variant/number of participants stating the inclusion of the relevant gene on their panel.
^ Based on Li *et al* (2017) Joint consensus recommendations from the Association for Molecular Pathology, American Society of Clinical Oncology and College of American Pathologists.[1] Variant classification by participants utilising alternative systems may have been aligned to the equivalent Li *et al* category (if available/applicable). Variant classification breakdowns are not equal to the sum of the total number of participants reporting the variant in any given gene as one participant did not provide variant classification information.
* Results returned by participants, at both the DNA and protein level, may have been harmonised to the equivalent Human Genome Variation Society (HGVS) approved nomenclature (http://varnomen.hgvs.org/) during the compilation of 'All Participants' results table. Protein nomenclature includes parenthesis as it represents a prediction from analysis at the DNA level. Please contact UKNEQAS LI for reference sequence information.
+ Descriptive statistics calculated for any variant with >2 quantification data points. Percentage values quoted have been subjected to rounding up/down to 1 dp.
¥ One participant reported a DNA sequence description of c.949dup, with a protein level description of p.Gln317Argfs*20. Given the consensus DNA description, this was considered to be a protein HGVS nomenclature error.

Your performance

Performance	Performance Status for this sample	Performance Status Classification Over 12 Month Period	
		Satisfactory	Critical
n/a	n/a	n/a	n/a

Please note: this programme is not currently performance monitored. We will work towards a performance monitoring system as the programme develops.

Methods

Please note figures in the tables below may not tally with the total number of participants returning results due to some participants not returning all data requested or using multiple techniques.

NGS platform used

	Returns
Illumina MiSeq	15
Illumina NextSeq	13
Illumina NovaSeq (no further information provided)	3
Illumina NovaSeq 6000	2
Illumina MiniSeq	2
ThermoFisher Scientific (Life Tech) Ion S5	2

NGS panel description

	Returns
Custom commercially developed	18
Illumina Trusight Myeloid Sequencing Panel	2
Illumina AmpliSeq™ Myeloid Panel	1
Illumina TruSight Oncology 500 High Throughput Panel	1
Illumina TruSight Oncology 500 (no further information provided)	1
ThermoFisher Scientific Oncomine Myeloid Research Panel	1
ThermoFisher Scientific Oncomine Lymphoma Panel	1
Archer VariantPlex Myeloid Panel	1
Qiagen Myeloid Neoplasms Panel	1
Sophia Genetics NGS Haloplex	1
Twist Bioscience Panel	1
Fluidigm 48x48 Access Array	1
Integrated DNA Technologies Panel	1
Oxford Gene Technology SureSeq CLL+CNV panel	1
In-house Panel	1
Other	4

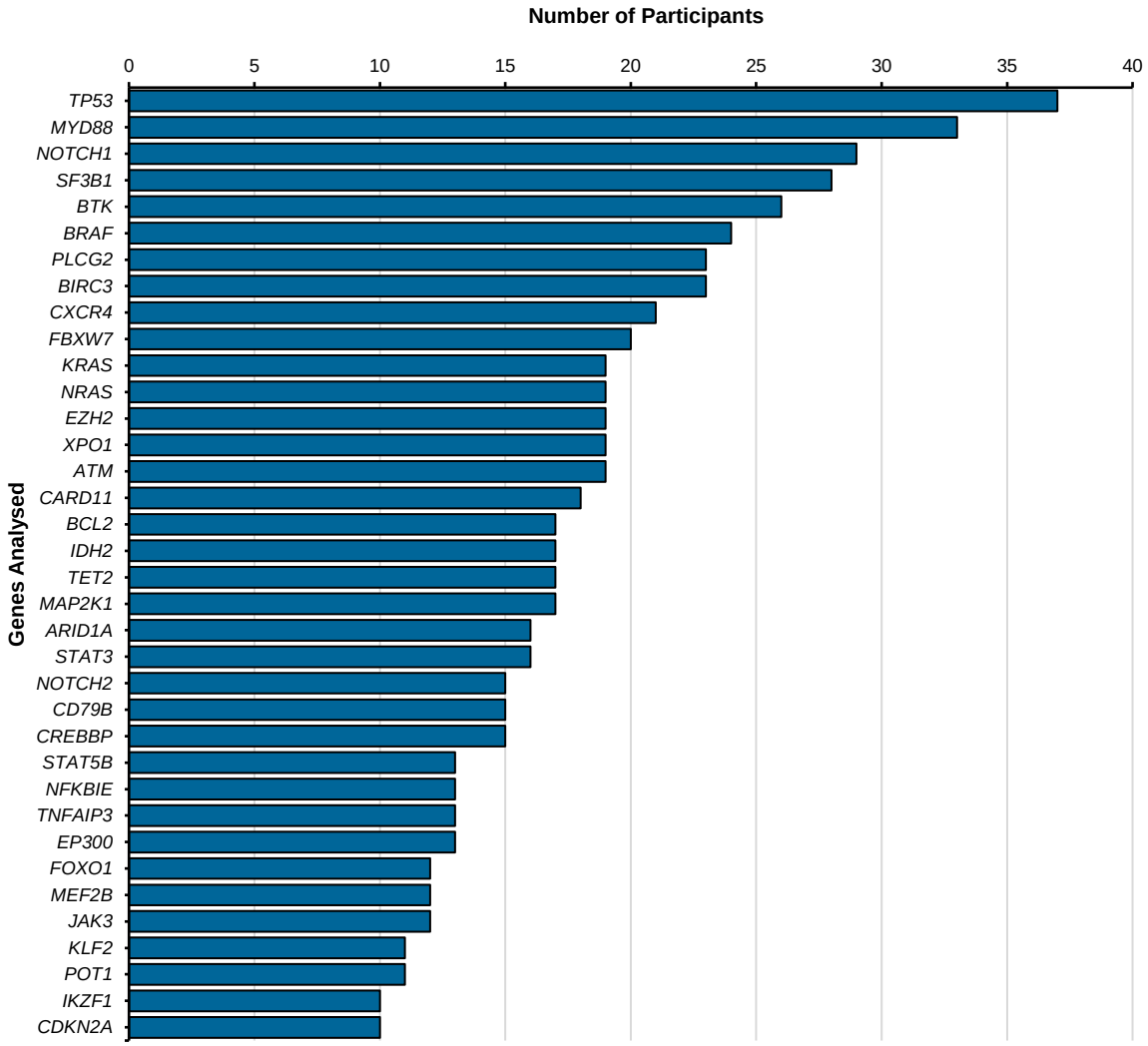

Figure 1: Histogram depicting genes routinely **analysed** by participants. Only genes routinely analysed by ≥10 participants are recorded in the histogram.

Annotation database resources

	Returns
COSMIC (Catalogue Of Somatic Mutations In Cancer)	36
ClinVar (NCBI)	32
The TP53 Database hosted by NCI (previously IARC TP53 database)	28
dbSNP (Short Genetic Variations, NCBI)	28
The Genome Aggregation Database (gnomAD)	22
My Cancer Genome (Vanderbilt-Ingram Cancer Center)	13
OncoKB	13
The Clinical Knowledgebase (CKB) Jackson Laboratory	12
HGMD (The Human Gene Mutation Database)	9
OMIM (NCBI)	8
Seshat	6
The Cancer Genome Atlas (TCGA)	5
VarSome	2

As stated by ≥2 participants.
Note: ERIC recommendations for *TP53* variant analysis in chronic lymphocytic leukaemia[2] advocate the use of the *TP53* Database hosted by NCI (previously IARC *TP53* database)[3], the UMD *TP53* database[4], Seshat[5], COSMIC[6] and ClinVar[7]. For this trial, no participants reported the use of the UMD *TP53* database.

Large-scale sequencing project dataset(s) routinely consulted during variant interpretation

	Returns
The Exome Aggregation Consortium (ExAC)	25
1000 Genomes	23
The Genome Aggregation Database (gnomAD)	9
NHLBI-GO Exome Sequencing Project (ESP)	8

As stated by ≥2 participants.

Published guideline(s) and/or recommendation(s) referenced to inform classification of somatic variant clinical significance/pathogenicity (in a Haemato-Oncology context)

	Returns
Li, M.M. *et al.* Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer. *J Mol Diagn.* 19(1):4-23 (2017).	24
Froyen, G. *et al.* Standardization of Somatic Variant Classifications in Solid and Haematological Tumours by a Two-Level Approach of Biological and Clinical Classes: An Initiative of the Belgian ComPerMed Expert Panel. *Cancers (Basel)*. 11(12): 2030 (2019).	10
Sukhai, M.A. *et al.* A classification system for clinical relevance of somatic variants identified in molecular profiling of cancer. *Genet Med.* 18(2):128–136 (2016).	3
Richards, S. *et al*. 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(5):405-424 (2015).	4

As stated by ≥2 participants.

Genome Assembly

	Returns
GRCh37/hg19	29
GRCh38	4

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

	Returns
10%	1
5%	21
4%	3
≥2-3%	10
1-<2%	2

Trial Comments

Methodology

- The vast majority of participants employed bridge amplified reversible dye terminator-based platforms from Illumina (n=35 data returns, 94.6% of participants).
- Five participants utilised a myeloid based panel in this trial distribution.
- Of the 33 laboratories providing information regarding genome assembly, 29 participants referenced GRCh37/hg19 (various minor releases/patches). Four participants referenced the GRCh38/hg38 genome-based assembly. At the time of reporting, GRCh38.p14 (equivalent to the UCSC hg38) is the latest human genome release (6th April 2022) from NCBI Genome Data Viewer (https://www.ncbi.nlm.nih.gov/genome/gdv/).
- The minimum Variant Allele Frequency (VAF) quoted for reporting variants ranged from 1% - 10%, with a median of 5%.
- The median minimum acceptable coverage (read depth) was 250x (range 5-1000x).
- All participants (n=37) provided information relating to the number of genes analysed on the NGS panel. A total of 195 different genes were present on participant NGS panels. The median number of genes tested on a given panel by laboratories for sample Lymphoid GP 105 was 24 (range 1-137).
- The most commonly sequenced genes on panels were: *TP53* (37 participants, 100% returns), *MYD88* (33 participants, 89.2% participant returns), *NOTCH1* (29 participants, 78.4% returns), *SF3B1* (28 participants, 75.7% returns) and *BTK* (26 participants, 70.3% returns).

Sample Lymphoid GP 105

Overall, 35/37 (94.6%) participants returning results for this trial stated detection of at least one DNA sequence variant in sample Lymphoid GP 105.

Classification of variants in this trial was largely in line with somatic variant classifications outlined in Li *et al.,* (2017) guidelines[1]. One participant appeared to report variant pathogenicity based on the somatic variant classifications outlined in Froyen *et al.,* (2019) guidelines[8] and one utilised the Richards *et al*., (2015) germline classification guidelines[9]. For clarity, variant classifications in this dataset have been aligned to Li *et al.,* (2017) joint consensus recommendations from the Association for Molecular Pathology, American Society of Clinical Oncology and College of American Pathologists[1] (where possible). This classification system utilises a tier system from I-IV, ranging from variants of strong, potential, or unknown clinical significance and also includes benign/likely benign variants. **This is the current preferred variant classification system when considering somatic variant interpretation. Please note for the purposes of this EQA programme, we only require the reporting of variants of strong, potential, or unknown clinical significance. Variants considered benign or likely benign do not need to be reported.**

In total, 35/37 (94.6%) participants that analysed *TP53* reported the NM_000546.6:c.949dup p.(Gln317Profs*20) variant. Of the 34 participants reporting the consensus variant, 28 (80.0%) participants classified the variant as of strong clinical significance. Five (14.3%) participants classified the variant as of potential clinical significance. One (2.9%) participant classified the variant as of unknown clinical significance and one (2.9%) did not provide a variant classification. The two participants that did not report the presence of a *TP53* variant in sample Lymphoid GP 105 reported detection of no variants in the sample.

- The median VAF reported for this variant was 97.3% with an interquartile range of 7.5% and a median read depth of 1,202x coverage.
- Seven participants reported the variant as NM_00546.6:c.949dupC. It should be noted that HGVS recommendations do not endorse the listing of the duplicated nucleotides as this creates a longer description with redundant information.
- This specific variant has not been previously reported in the *TP53* database (version R20)[3], however, a c.949_950insN p.Glu317fs variant is listed, describing the same predicted protein change as the c.949dup variant (p.Glu317fs). The *TP53* database reports a COSMIC reference (COSV52867051)[6], which describes a c.949dup p.Glu317Profs*20 variant, reported in association with head and neck carcinomas.
- The variant is absent from both the UMD[4] or Seshat[5] *TP53* databases.
- Whilst this variant is absent from the UMD[4] database, there are several frameshift variants (14 unique variants reported across 38 different samples with unique UMD identifiers) reported in the database resulting from deletion or insertion of nucleotides either at or encompassing position c.949. These frameshift variants have been observed across a range of tumour types, including one report from chronic lymphocytic leukaemia.

- This variant has been identified by Malcikova *et al*., (2021) in a study assessing the clinical impact and clonal evolution of low-burden *TP53* mutations in CLL in relation to different treatment options[10].
- For the predicted protein change associated with the *TP53* variant; there was variable use of the HGVS nomenclature, as outlined in the table below.

Protein nomenclature *TP53* variant	n	Comments
p.(Gln317Profs*20)	16	Compliant with HGVS recommendations. Parentheses reflect the analysis of DNA and the predicted status of the protein level description. * or Ter are equally acceptable to indicate a termination/STOP codon. Similarly, the short description of a frameshift variant, p.(Gln317fs), would also be compliant.
p.(Gln317ProfsTer20)	8	
p.Gln317ProfsTer20	4	Mostly compliant with HGVS recommendations; however, parentheses are required in this context as DNA has been analysed, thus any protein change is only predicted based on the DNA variant detected. * or Ter are equally acceptable to indicate a termination/STOP codon.
p.Gln317Profs*20	1	
p.Q317Pfs*20	2	Mostly compliant with HGVS recommendations; however, parentheses are required in this context as DNA has been analysed, thus any protein change is only predicted based on the DNA variant detected. Three letter amino acid code is preferred when describing protein changes.
p.(Q317Pfs*20)	1	Mostly compliant with HGVS recommendations; however, three letter amino acid code is preferred when describing protein changes.
(p.Gln317ProfsTer20)	1	Mostly compliant with HGVS recommendations; however, there is incorrect use of parentheses in this description.
p.(Gln317fs*20)	1	Frameshifts can be described using a short format, however, should not include any further detail other than the first amino acid changed, its position and “fs”. For this variant, the short format would be p.(Gln317fs).
p.Gln317Argfs*20	1	Frameshift variant descriptions should start with the first amino acid changed. The first codon affected by a variant is Gln317, resulting in a substitution for Proline (Pro) and not Arginine (Arg) as described here.

Colour coding reflects the level of compliance with current HGVS recommendations (v20.05)[11,12]: **green** = fully compliant, **amber** = generally compliant with some omission(s)/minor issue(s) and **red** = nomenclature error(s)/ fails to comply with the recommendations.

Twelve out of 18 participants (66.7%) routinely analysing *CARD11* in the context of lymphoid neoplasms identified the NM_032415.7:c.1222C>T p.(Arg408Cys) variant within exon 9. Of the 12 participants reporting the variant, nine (75.0%) participants classified the variant as of unknown clinical significance. Three participants (25.0%) classified the variant as of potential clinical significance. Of the six participants that failed to detect the variant, three indicated that full coverage was achieved across the region of this variant. One participant did not sequence exon 9 of the *CARD11* gene, one detected no reportable variants within sample Lymphoid GP 105 and one participant provided no information relating to sequence coverage or internal quality control.

- The median VAF reported for this variant was 38.2% with an interquartile range of 3.3% and a median read depth of 5,465x coverage.
- This variant is present in the COSMIC database (COSV67797339)[6] in association with endometrioid and adenocarcinomas.
- HGVS nomenclature for this *CARD11* variant was largely in accordance with the recommendations for protein descriptions. 10/12 (83.3%) participants described the predicted amino acid change as p.(Arg408Cys), one described the variant as p.Arg408Cys and one as p.R408C. Parentheses are usually required in this context as genomic DNA is conventionally analysed; thus, any protein change is only predicted based on the DNA variant detected. Furthermore, three letter amino acid code is preferred when describing protein changes.

Four out of five participants (80.0%) routinely analysing *CCND3* in the context of lymphoid neoplasms identified a NM_001760.5:c.860T>A p.(Val287Asp) variant. Two participants reported the variant as of potential clinical significance and two as of unknown clinical significance. The participant routinely analysing *CCND3* who failed to detect the variant reported that full coverage was achieve. The remaining participants provided no information relating to sequence coverage or internal quality control.

- The median VAF reported for this variant was 51.0% with an interquartile range of 1.7% and a median read depth of 2,327x coverage.
- This variant has been reported three times in the COSMIC database (COSV57827926)[6] in association with lymphoid neoplasms (twice in diffuse large B cell lymphoma and one unspecified).
- Three participants described the predicted amino acid change as p.(Val287Asp) and one participant described the variant as p.Val287Asp. Parentheses are usually required in this context as genomic DNA is conventionally analysed; thus, any protein change is only predicted based on the DNA variant detected.

Three out of five participants routinely analysing *PTPRD* in the context of lymphoid neoplasms identified a NM_002839.4:c.2321A>T p.(Gln774Leu) variant in exon 25. Two participants reported the variant as of unknown clinical significance and one as of potential clinical significance. One participant routinely analysing *PTPRD* but not detecting this variant stated that their region of interest was limited to exons 35-46. The remaining participant provided no information relating to their region of interest, sequence coverage or internal quality control.

- The median VAF reported for this variant was 66.0% with an interquartile range of 1.7% and a median read depth of 1,526x coverage.
- There is a single report of this variant in the COSMIC[6] database in association with lung adenocarcinoma.
- The variant is not present in either dbSNP[13] or gnomAD[14] databases.
- Two participants described the predicted amino acid change as p.(Gln774Leu) and one reported it as p.Gln774Leu. Parentheses are usually required in this context as genomic DNA is conventionally analysed; thus, any protein change is only predicted based on the DNA variant detected.

Two out of eight participants routinely analysing *RPS15* in the context of lymphoid neoplasms identified a NM_001018.5:c.217C>T p.(Pro73Ser) variant. Both participants reported the variant as of unknown clinical significance and described it using HGVS recommended nomenclature. Of the six participants routinely analysing *RPS15* who failed to detect the variant, four reported that full coverage was achieve. One participant stated that the full gene was sequenced but provided no information relating to coverage or internal quality control. One participant provided no information relating to their region of interest, sequence coverage or internal quality control.

- The two participants reported a VAF of 10.0 and 21.0%.
- This variant is reported in COSMIC[6] in association with adenocarcinomas and squamous cell carcinomas.
- The variant is also present in dbSNP[13] (rs146047499) and the gnomAD[14] database at low frequency (<0.1%).

Thank you to all participants for their continued engagement with the Lymphoid Gene Panels Programme. The valuable methodological information supplied, including details regarding panel region of interest (ROI) and related reference sequences, facilitates an informative trial report. Please do contact us if you have any suggestions regarding how this pilot programme could be improved for future trial distributions: admin@ukneqasli.co.uk.

It is beyond the scope of this programme to comment conclusively on the clinical significance of the variants reported by participants. We acknowledge the limitations of this EQA exercise.

Please note: The information provided herein is for participant information only. Clinical decision making with regards to variant interpretation, pathogenicity, actionability and predicted disease outcomes should not be based solely on comments provided by UK NEQAS LI in this EQA trial report.

References

1. Li, M. M. *et al.* 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 (2017).

2. Malcikova, J. *et al.* ERIC recommendations for TP53 mutation analysis in chronic lymphocytic leukemia - Update on methodological approaches and results interpretation. *Leukemia* **32**, 1070–1080 (2018).

3. The *TP53* Database (R20, July 2019). Available at https://tp53.isb-cgc.org. (Accessed: 6th February 2023).

4. Leroy, B., Anderson, M. & Soussi, T. TP53 Mutations in Human Cancer: Database Reassessment and Prospects for the Next Decade. *Hum. Mutat.* **35**, 672–688 (2014). Database available at: https://p53.fr/

5. Tikkanen, T. *et al.* Seshat: A Web service for accurate annotation, validation, and analysis of TP53 variants generated by conventional and next-generation sequencing. *Hum. Mutat.* **39**, 925–933 (2018). Database available at: http://vps338341.ovh.net/

6. Catalogue of Somatic Mutation in Cancer (COSMIC). Available at: https://cancer.sanger.ac.uk/cosmic. (Accessed: 19th January 2023).

7. Landrum, M. J. *et al.* ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res.* **46**, D1062–D1067 (2018).

8. Froyen, G. *et al.* Standardization of Somatic Variant Classifications in Solid and Haematological Tumours by a Two-Level Approach of Biological and Clinical Classes: An Initiative of the Belgian ComPerMed Expert Panel. *Cancers* **11**(12): 2030, (2019).

9. Richards, S. *et al*. 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**, 405-423 (2015).

10. Malcikova, J. *et al*. Low-burden TP53 mutations in CLL: clinical impact and clonal evolution within the context of different treatment options. *Blood*. **138**(25), 2670-2685 (2021).

11. Human Genome Variation Society (HGVS). Available at: https://varnomen.hgvs.org/. (Accessed: 19th January 2023).

12. den Dunnen, J. T. *et al.* HGVS Recommendations for the Description of Sequence Variants: 2016 Update. *Hum. Mutat.* **37**, 564–569 (2016).

13. The Single Nucleotide Polymorphism Database (dbSNP) of Nucleotide Sequence Variation. Available at: https://www.ncbi.nlm.nih.gov/snp. (Accessed: 19th January 2023).

14. Genome Aggregation Database (gnomAD). Available at: http://gnomad.broadinstitute.org/. (Accessed: 19th January 2023).

Information with respect to compliance with standards BS EN ISO/IEC 17043:2010

4.8.2 a) The proficiency testing provider for this programme is:
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4.8.2 b) The coordinators of UK NEQAS LI programmes are Mr Liam Whitby (Director) and Mr Stuart Scott (Centre Manager).

4.8.2 c) Person(s) authorizing this report:
Mr Liam Whitby (Director) or Mr Stuart Scott (Centre Manager) of UK NEQAS LI.

4.8.2 d) Pre issue testing of samples for this programme is subcontracted, although the final decision about sample suitability lies with the EQA provider; no other activities in relation to this EQA exercise were subcontracted. Where subcontracting occurs it is placed with a competent subcontractor and the EQA provider is responsible for this work.

4.8.2 g) The UK NEQAS LI Confidentiality Policy can be found in the Quality Manual which is available by contacting the UK NEQAS LI office. Participant details, their results and their performance data remain confidential unless revealed to the relevant NQAAP when a UK participant is identified as having performance issues.

4.8.2 i) All EQA samples are prepared in accordance with strict Standard Operational Procedures by trained personnel proven to ensure homogeneity and stability. Where appropriate/possible EQA samples are tested prior to issue. Where the sample(s) issued is stabilised blood or platelets, pre and post stability testing will have proved sample suitability prior to issue.

4.8.2 l), n), o), r) & s) Please refer to the UK NEQAS LI website at www.ukneqasli.co.uk for detailed information on each programme including the scoring systems applied to assess performance (for BS EN ISO/IEC 17043:2010 accredited programmes only). Where a scoring system refers to the 'consensus result' this means the result reported by the majority of participants for that trial issue. Advice on the interpretation of statistical analyses and the criteria on which performance is measured is also given. Please note that where different methods/procedures are used by different groups of participants these may be displayed within your report, but the same scoring system is applied to all participants irrespective of method/procedure used.

4.8.2 m) We do not assign values against reference materials or calibrants.

4.8.2 q) Details of the programme designs as authorized by The Steering Committee and Specialist Advisory Group can be found on our website at www.ukneqasli.co.uk. The proposed trial issue schedule for each programme is also available.

4.8.2 t) If you would like to discuss the outcomes of this trial issue, please contact UK NEQAS LI using the contact details provided. Alternatively, if you are unhappy with your performance classification for this trial, please find the appeals procedure at www.ukneqasli.co.uk/contact-us/appeals-and-complaints/

4.8.4) The UK NEQAS LI Policy for the Use of Reports by Individuals and Organisations states that all EQA reports are subject to copyright, and, as such, permission must be sought from UK NEQAS LI for the use of any data and/or reports in any media prior to use. See associated policy on the UK NEQAS LI website: http://www.ukneqasli.co.uk/eqa-pt-programmes/new-participant-information/