UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Pilot Myeloid Gene Panels (Not Accredited) - INTERIM REPORT

Distribution – 252601
Date Issued – 22 Dec 2025

Participant –
Closing Date – 13 Feb 2026

IMORTANT NOTICE: Please accept our apologies for the delay in publication of the Myeloid Gene Panels 242501 trial report educational addendum (sample Myeloid GP 118). We are collaborating with the HGVS Variant Nomenclature Committee (HVNC) to establish the most appropriate application of ISCN (International System for Human Cytogenomic Nomenclature) and HGVS (Human Genome Variation Society) Nomenclature in relation to *KMT2A* Partial Tandem Duplication (PTD).

Trial Comments

This trial was issued to 150 participants; 136 (90.7%) laboratories have returned results. Of the 14 participants failing to provide results at the time of trial analysis, one laboratory pre-notified us extenuating circumstances and a further two participants have a late submission request extension in place.

Sample Comments

The lyophilised trial sample (Myeloid GP 120) was formulated from the peripheral blood of a patient with a working diagnosis of acute myeloid leukaemia (AML) (no further clinical details available) and distributed by UK NEQAS LI. The material was potentially processed >48 hours following collection.

Your Myeloid Gene Panels Laboratory Record status for this trial:	

IMPORTANT: To permit meaningful trial data analysis it is essential the information held in your Laboratory Record is complete and accurately reflects your current practice in relation to this programme. Please provide all the information as requested and/or check it carefully to ensure methodological details are up to date when requested to do so.

Sample Myeloid GP 120

Your Results – Variant(s) of strong clinical significance

Gene	Your DNA sequence variant detected	Your protein variant

Your Results – Variant(s) of potential clinical significance

Gene	Your DNA sequence variant detected	Your protein variant

Your Results – Variant(s) of unknown clinical significance

Gene	Your DNA sequence variant detected	Your protein variant

Please note, due to formatting limitations some rows may appear blank within the tables(s) above. All submitted variant(s) of unknown clinical significance may not be reflected in the above table for individual participants due to formatting and space constraints.

All Participant Results

In the interests of clarity, we have only summarised variants reported by ≥5 participants in the table below.

Gene	n [a]	Variant classification [b] Clinical Significance:			Variant detected (consensus) [c]		Median VAF % (IQR) [d]
		Strong	Potential	Unknown	DNA sequence (transcript) description	Protein level description	
IDH1	135/136[e]	132	2	0	NM_005896.4:c.394C>G	p.(Arg132Gly)	48.0 (3.4)
NPM1	136/136[f]	134	1	0	NM_002520.7:c.863_864insCCTG[g]	p.(Trp288Cysfs*12)[g]	42.4 (10.6)
DNMT3A	135/135	110	23	0	NM_022552.5:c.2645G>A[h]	p.(Arg882His)[h]	48.0 (2.3)
FLT3	131/135	125	3	2	NM_004119.3:c.1801_1802insGGGATTTCAGAGAATATGAATATGATC	p.(Asp600_Leu601insArgAspPheArgGluTyrGluTyrAsp)	41.8 (10.0)
					Atypical 'Internal Tandem Duplication' ITD (27 bp)[i]		
EP300	6/19	0	1	5	NM_001429.4:c.316A>G	p.(Ser106Gly)	50.0 (3.3)

[a] Total number of participants reporting this variant/number of participants stating the inclusion of the relevant gene on their panel or known to feature the gene on their panel due to identification of the consensus variant (or other variant in the gene). Please note for this trial 4 returning participants failed to provide full Laboratory Record information. Not all laboratories provided sufficient gene/region of interest information for their panel to permit identification of all false negative results in the data set. Additionally, participant(s) may also have reported a consensus variant from a gene not stated as included on their panel.
[b] 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]. Laboratories submitting multiple classifications for the same variant are excluded from this section of the table; therefore, the total number of classifications may not equal the total participants detecting the variant.
[c] Nomenclature provided in the table is based on the MANE Select/Plus Clinical (v1.4)[2] reference transcript and genome build GRCh38, unless specified. Please refer to the comments section (in the finalised educational addendum) for further information about reference sequences. Results returned by participants, at both the DNA and protein level, may have been harmonised to the equivalent Human Genome Variation Society (HGVS) Nomenclature specification (version 21.3)[3-5] during the compilation of the 'All Participant Results' table. Information regarding a variant(s) reported in any gene listed in the table, which could not be identified as equivalent to a consensus variant has been excluded. Protein nomenclature includes parentheses as it represents a prediction from analysis at the DNA level.
[d] Descriptive statistics calculated for any variant with >2 quantification data points. Median VAF calculated for DNA based assays (where input material was stated), all panels and platforms. Percentage values quoted have been subjected to rounding up/down to 1 d.p., IQR = interquartile range. Quantitative data points may have been excluded from the statistics if the associated nomenclature provided was considered equivocal.
[e] Includes a single laboratory returning the *IDH1* variant with a suspected nomenclature typographical error at the DNA level, NM_005896.4:c.394C>A (the result was in consensus at the protein level). Additionally, an excluded participant returned in consensus *IDH1* variant nomenclature at the DNA level but inappropriately included an *NPM1* related protein description (a reference sequence was not cited).
[f] Includes five laboratories submitting various nomenclature errors of note at the DNA level (NM_002520.7), including c.863_864insCC, c.860_863dup, c.863_864ins, c.860_863dupTCTG and c.860_861insCTGC. The variant descriptions provided at the protein level were in consensus.
[g] Alternative transcript description (isoform 2) - NM_199185.3:c.776_777insCCTG p.(Trp259Cysfs*12).
[h] Alternative transcript description (isoform b) - NM_153759.3:c.2078G>A p.(Arg693His).
[i] The various nomenclature approaches taken by participants for this atypical *FLT3* ITD type variant, which appears to include additional nucleotides (preventing description as a duplication event), will be discussed in the subsequently issued educational addendum.

Your Performance

Performance	Performance Status for this Sample	Performance Status Classification Over 3 Sample 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 scoring 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. At the time of reporting 4 returning participants failed to provide the minimum Laboratory Record information requested.

Methodological approach

	Returns
Targeted Gene Panel (DNA seq)	120
Targeted Gene Panel (DNA with RNA fusion transcript seq)	11
Other	2

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

	Returns
Illumina MiSeq	39
Illumina NextSeq 1000/2000	20
Illumina NextSeq 550	18
Thermo Fisher Scientific Ion Torrent Genexus	14
Illumina NovaSeq 6000	13
Thermo Fisher Scientific (Life Tech) Ion S5	10
Illumina MiniSeq	8
Thermo Fisher Scientific (Life Tech) Ion S5 Plus	6
Illumina NovaSeq X	4
Illumina NovaSeq X Plus	2
Thermo Fisher Scientific (Life Tech) Ion S5 Prime	2
Element Bioscience AVITI	2
MGI DNBSEQ-G400	2
Illumina NovaSeq	1

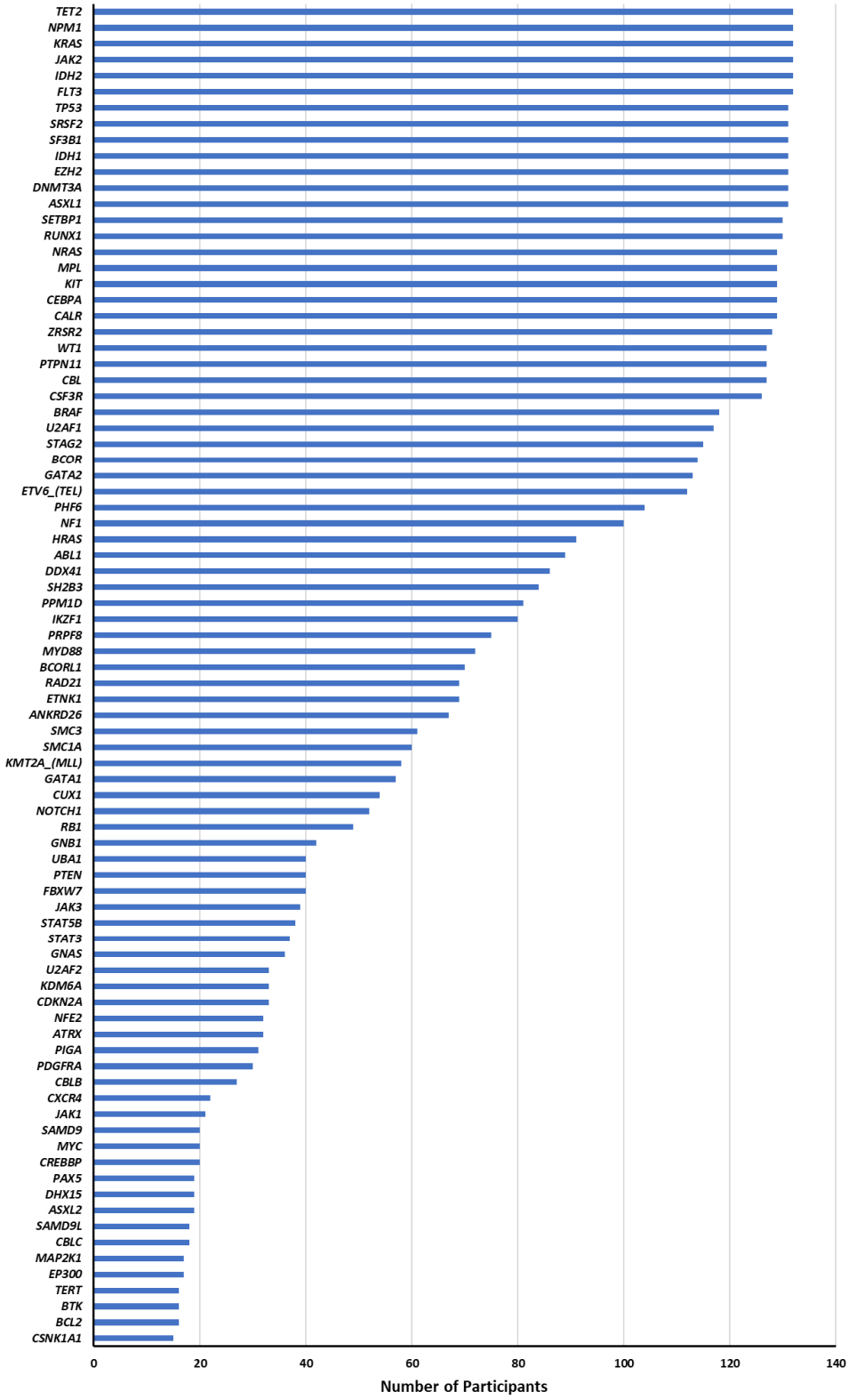
NGS panel description (to analyse the sample in this trial)

	Returns
Oncomine Myeloid Research Assay	16
Sophia Genetics Myeloid Solution (MYS)	15
Oncomine Myeloid Assay GX v2	14
In house (capture based)	14
Agilent SureSelect Custom XT HS2 Panel	13
Twist Custom Panel	10
Qiagen QIASeq Custom Panel	8
Archer VARIANTPlex Myeloid Panel	5
In house (amplicon based)	5
Sophia Genetics [Myeloid Solution] (CMYS) Custom Panel	5
AmpliSeq for Illumina Myeloid Panel	4
Archer VARIANTPlex Core Myeloid Panel	2
Agilent SureSelect Custom QXT Panel	2
Illumina TruSight Oncology 500 (TSO500) Panel	2
IDT Custom Panel (capture-based)	2
Health in Code Haematology OncoKitDx	2
Sophia Genetics Myeloid Solution (MYS) - Custom Extended	2

As stated by ≥2 participants.

Genes routinely analysed by participants (in this clinical context).

Information provided by 132 laboratories; data is presented as submitted by participants (and not subject to comprehensive cross checking with reference to variant(s) detected results from individual laboratories). Only genes routinely analysed by at least 15 participants are represented in the chart.



Genome Assembly

	Returns
GRCh37/hg19	105
GRCh38	28

Minimum variant allele frequency (VAF) for reporting identification of a deletion/duplication/insertion variant or 'indel' variant

	Returns
0.5%	2
1%	9
2%	22
2.5%	8
3%	11
3.5%	1
4%	5
4.5%	1
5%	72
10%	2

Minimum variant allele frequency (VAF) for reporting identification of a single nucleotide variant (SNV) or substitution variant

	Returns
0.5%	1
1%	16
2%	23
2.5%	8
3%	15
3.5%	1
4%	5
4.5%	1
5%	63

Annotation database resources

	Returns
ClinVar (NCBI)	114
COSMIC (Catalogue Of Somatic Mutations In Cancer)	98
TP53 Database (National Cancer Institute) previously hosted by WHO IARC	69
OncoKB (Memorial Sloan Kettering Cancer Center)	39
OMIM (NCBI)	34
Cancer Hotspots (Memorial Sloan Kettering Cancer Center)	33
cBioPortal (Memorial Sloan Kettering Cancer Center et al.)	33
Seshat (TP53) Database	33
My Cancer Genome (Vanderbilt-Ingram Cancer Center)	21
HGMD (The Human Gene Mutation Database)	20
The Cancer Genome Atlas (TCGA)	17
Clinical Knowledgebase (CKB) Jackson Laboratory[a]	18
UniProt (EMBL-EBI, SIB, PIR)	15
CIViC (Clinical Interpretation of Variants in Cancer)	13
UMD (Universal Mutation Database) TP53 Database	13
PeCan Pathogenicity Information Exchange (PIE) (St Jude)	6
Molecular Tumor Board Portal (MTB) (Karolinska Institutet)	4

As stated by ≥2 participants.

[a] Acquired and hosted by Genomenon, operating as CKB CORE (public access) and CKB BOOST (subscription).

Large population dataset/resources routinely consulted

	Returns
gnomAD (Genome Aggregation Database)	119
dbSNP (Short Genetic Variations, NCBI)	75
1000 Genomes	45
ESP (Exome Sequencing Project, NHLBI GO)	23

As stated by ≥2 participants.

***In silico* tools utilised to predict impact on splicing**

	Returns
Splice AI	68
SpliceSiteFinder	28
GeneSplicer	24
MaxEntScan	24
NNSPLICE	22

As stated by ≥2 participants.

Aggregation tool(s) utilised to access annotation resources

	Returns
Franklin (GENOOX)	69
Varsome (SAPHETOR)	63
Alamut (SOPHiA GENETICS)	47
QCI Interpret (Qiagen)	7
Mobidetails (MoBiDiC, Montpellier University Hospital)	7

As stated by ≥2 participants.

Trial Comments

Methodology

- The majority of returning participants (with the relevant information provided in their Laboratory Record) described the application of a DNA based targeted gene panel next generation sequencing (NGS) testing approach (n=131). At least 11 laboratories stated the additional inclusion of fusion gene transcript sequencing. Please note, for this programme laboratories are not requested to report large changes affecting genome architecture or copy number variants (>50 kb).
- The average number of genes currently analysed by laboratories on a given panel is 52 (range 25-136). For the genes most frequently included on participant gene panels (and analysed in this clinical context) please refer to the chart on page 6.
- Overall, 73.5% (n=100) returning participants providing the relevant information employed a bridge amplified reversible dye terminator-based platform(s) from Illumina to analyse sample Myeloid GP 120. The remaining laboratories stated the use of ThermoFisher Scientific (Ion Torrent) (n=31), MGI DNBSEQ (n=2) or Element Bioscience (n=2) technology. Some centres utilised more than one instrument from the same (n=6) or different (n=2) manufacturer.
- The most utilised ‘off the shelf’ commercially available panel kits included the ThermoFisher Scientific Oncomine Myeloid Research Panel (n=16), Sophia Genetics Myeloid Solution (n=15), ThermoFisher Scientific Oncomine Myeloid Assay GX v2 (n=14) and Archer VARIANTPlex Myeloid Panel (n=5).

- For a deletion/duplication/insertion event, 44.4% (n=59) of participants quoted a minimum threshold for reporting of <5% VAF. This is an increased compared to 39.3% of participants for the previous trial (Myeloid GP 252402).
- For a SNV the thresholds for reporting continue to be set lower with 52.6% (n=70) laboratories applying a minimum VAF <5% for this trial. Again, this is an increase from 47.1% of participants for the previous trial (Myeloid GP 252402).

Annotation and interpretation

- The proportion of participants known to be working to the GRCh38 human genome assembly was 21.1% (n=28).
- ClinVar (n=114), COSMIC (n=98) and gnomAD (n=119) remain the resources most widely utilised by participants. The list of databases and tools accessed by centres continues to expand, please refer to the tables on pages 8-9 for further information.
- The *in silico* tools utilised most frequently to predict impact on splicing included Splice AI (n= 68) and are summarised in the table on page 10.
- The adoption of aggregation tools incorporating AI powered interpretation, such as Franklin (GENOOX/Qiagen) (n=69), continues to grow. AI models and training datasets may not be subject to a level of curation sufficient for clinical diagnostic application and should be utilised with caution. Diligent verification/validation by laboratories implementing such tools is required. It is prudent to check the underpinning publication(s) and/or supporting source information carefully. Many resources access the same primary dataset(s); laboratories are encouraged to be mindful of duplicated/misrepresented evidence when classifying variants in terms of biological and/or clinical significance.
- The Association for Clinical Genomic Science (ACGS) guidelines for the classification of oncogenicity of somatic variants in cancer: recommendations by the UK somatic variant interpretation group (SVIG-UK) have very recently been published[6].

Sample Myeloid GP 120

All returning participants (n=136) reported at least one sequence variant in sample Myeloid GP 120 (please refer to the summary 'All Participant Results' table on page 3 for further details).

One laboratory employing OGT SureSeq Myloid Plus (Illumina MiniSeq) also reported an out of consensus *ASXL1* c.1934del p.(Gly645ValfsTer58) (VAF = 4.4%) variant (referencing NM_015338) and classified it as of strong clinical significance.

Further details of relevant gene panel scopes, a breakdown of nomenclature and the discussion of educational aspects relating to variant interpretation will be subsequently published in a Myeloid Gene Panels 252601 report addendum to form the finalised trial report. Participants will be notified by email as soon as the final trial report is available.

Final Remarks

Many thanks to those participants who provided their full Laboratory Record information, as requested. The valuable methodological information supplied, including details regarding panel region of interest (ROI) and related reference sequences, facilitates an informative trial report.

Continued growth in participation for this pilot programme has produced a large dataset requiring manual analysis and independent checking. We acknowledge that currently maintaining the Laboratory Record and standard trial data entry are laborious for participants. We will be reviewing data entry and reporting procedures in 2026-2027, this will include a consultation with stakeholders to collate feedback to inform the future direction and format of the Myeloid GP programme.

The consistent use of standardised nomenclature with an appropriate reference sequence is critical for the effective communication of genetic testing results across the literature/databases and within a clinical setting. We strongly urge participants to comply with the latest HGVS Nomenclature specifications[3] and utilise a uniquely identifiable transcript reference sequence(s) designated by the MANE collaboration[2].

Variant classifications have been aligned to Li *et al*., (2017) joint consensus recommendations from the Association for Molecular Pathology (AMP), American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP)[1]. The classification system utilises a tier-based system (I-IV): variants of strong, potential or unknown clinical significance and benign/likely benign variants. Please note for the purposes of this EQA programme, participants are not required to submit variants considered to be benign/likely benign (neutral).

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. The information provided herein is for participant information only. Clinical decision making with regards to variant interpretation, oncogenicity/pathogenicity (driver status), actionability and predicted disease outcomes should not be based solely on comments provided by UK NEQAS LI.

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

References

1. Li, MM *et al.* Standards and guidelines for the interpretation and reporting of sequence variants in cancer. *J Mol Diagn.* 19(1):4-23 (2017).

2. Morales, J *et al.* A joint NCBI and EMBL-EBI transcript set for clinical genomics and research. *Nature* 604:310–315 (2022).

3. Human Genome Variation Society (HGVS) Nomenclature, https://hgvs-nomenclature.org/stable/ (v21.1.3) – accessed March 2026.

4. Dunnen, JT *et al.* HGVS recommendations for the description of sequence variants: 2016 update. *Hum Mutat*. 37(6):564-9 (2016).

5. Hart, R *et al.* HGVS Nomenclature 2024: Improvements to community engagement, usability, and computability. On behalf of the HGVS Variant Nomenclature Committee (HVNC) *Genome Med.* 16:149 (2024).

6. Burghel, GJ *et al*. Association for Clinical Genomic Science (ACGS) guidelines for the classification of oncogenicity of somatic variants in cancer: recommendations by the UK somatic variant interpretation group (SVIG-UK). *J Med Genet.* 63(3):147-156 (2026).

Information with respect to compliance with standards BS EN ISO/IEC 17043:2023

7.4.3.2 a) The proficiency testing provider for this programme is:
UK NEQAS for Leucocyte Immunophenotyping
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7.4.3.2 b) Person(s) authorising this report: Mr Stuart Scott (Director) of UK NEQAS LI.

7.4.3.2 c) Administration and shipping for this programme is provided by EQA International Limited.

7.4.3.2 c) Pre issue and post closure testing of samples for this programme is externally provided, although the final decision about sample suitability lies with the EQA provider. Aside from the activities mentioned above, no other activities in relation to this EQA exercise were externally provided.

7.4.3.2 c) Where externally provided products or services are used in the delivery of EQA, a competent supplier is used, the EQA provider is responsible for this work and participants are informed accordingly.

7.4.3.2 f) The UK NEQAS LI Privacy Policy can be found at the following link: https://sheffield-ukneqas.ipassportqms.com/document_download/NjRlNTgxYzctMTI4ZS00MTg4LWI2ZDMtZDdkYzJhMTFlZTg3. Participant details, their results and their performance data remain confidential unless we are required by law to share this information. Where required by law or authorised by contractual arrangements to release confidential information, UK NEQAS LI will notify those concerned of the information released, unless prohibited by law. For UK participants, the relevant National Quality Assessment Advisory Panel (NQAAP) is informed when a UK participant is identified as having performance issues. Please note, the activities of the NQAAPs are currently paused, whilst alternative funding mechanisms are sought.

7.4.3.2 h) All EQA samples are prepared in accordance with strict standard operating procedures by trained personnel proven to ensure homogeneity and stability. Where appropriate/possible EQA samples are tested prior to issue.

7.4.3.2 j), m), n), o) & r) Please refer to the UK NEQAS LI website at www.ukneqasli.co.uk for detailed information on each programme including the design and implementation of the programme, example annotated reports including and the performance systems applied to assess performance (for BS EN ISO/IEC 17043:2023 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.

7.4.3.2 l) We do not assign values against reference materials or calibrants.

7.4.3.2 q) Details of the programme designs as authorised 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.

7.4.3.2 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/

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