Lung Cancer (NSCLC) [Tissue] Common Biomarkers EQA 2024

Post-appeals Summary Scheme Report

EMQN CIC
Unit 4, Enterprise House, Pencroft Way,
Manchester Science Park, Manchester M15 6SE
tel: +44.161.757.1591
email: office@emqn.org

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tel: +44 (0) 161 757 1591 **web:** www.emqn.org **mail:** office@emqn.org

Dear Colleague, 27th January 2025

This external quality assessment (EQA), Lung Cancer (NSCLC) [Tissue] Common Biomarkers is run by EMQN CIC. The EQA assessment included the scoring of genotype, interpretation and clerical accuracy. This EQA summary scheme report includes assessment data using harmonised marking criteria. EMQN CIC is responsible for this EQA, and all correspondence related to it should be directed to us.

The assessment is now complete and your individual laboratory scores have been agreed by the assessors. Please go to your EMQN CIC website account to download your Individual Laboratory Report (ILR):

- EMQN CIC (www.emqn.org): select the 2024 "Lung Cancer (NSCLC) [Tissue] Common Biomarkers" EQA.

EQA design and purpose

The aim of this EQA is to assess the testing accuracy (genotyping), and reporting (biological and clinical interpretation of the test result and overall report content and clerical accuracy) for Lung Cancer (NSCLC) [Tissue] Common Biomarkers and to help make improvements using a combination of assessment and educational feedback (expert commentary) via both individual laboratory reports (ILRs) and this EQA Scheme Summary Report when required.

The EQA design meets these objectives by assessing the ability of the participating laboratories to:

- Genotype sections from patient tumour and artificial FFPE samples accurately and to identify which variants are relevant to the clinical referral,
- Interpret the results in response to the clinical referral in a clear and concise format,
- Correctly use internationally accepted standard nomenclature, and
- Provide appropriate and accurate patient and sample identifiers.

This scheme report contains information from the cohort of participants including geographical spread, methodologies employed, common errors, learning points and scheme statistics to allow participants to benchmark their results.

Summary report on behalf of the assessment team

All Cases

Genotyping

- Testing of the *EGFR, BRAF* and *KRAS* genes was mandatory this year: 95.5% of participants tested and reported all three genes, 0.32% tested *EGFR* and *BRAF*, 0.32% tested *EGFR* and *KRAS* and 3.9% tested *EGFR* only.
- A total of 18 critical genotyping errors were made (see tables 6 and 8). The genotyping error rate was 1.18% (20/1528 genotypes), a significant improvement when compared to 1.88% in 2023. The mean genotyping score was 1.91 (1.89 in 2023).
- 83.0% of laboratories received full marks for genotyping across all five cases (75.7% in 2023).
- EMQN supports the use of MANE Select and MANE Plus Clinical as denoted by the MANE initiative, for the standardization of variant annotation, interpretation and reporting[1]. Support for Locus Reference Genomic (LRG) reference sequences has been discontinued. While use of LRG reference sequences is still acceptable, RefSeq or Ensembl transcripts specified by MANE are now preferred for sequence nomenclature. Laboratories have not been penalised for using LRG reference sequences this year.
- Laboratories should be aware of current HGVS nomenclature recommendations, particularly guidelines applicable to deletions and deletion-insertion (delins) nomenclature, and for describing uncertainty where assays which cannot distinguish between several variants at a particular codon have been used.

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- The caret "^" symbol can be used to indicate uncertainty where the exact variant cannot be determined by a test. Some laboratories used this symbol but not correctly according to HGVS 21.0.4 guidelines, see guidance for use of the caret symbol under https://hgvs-nomenclature.org/stable/recommendations/general/ and https://hgvs-nomenclature.org/stable/recommendations/uncertain/.
- Laboratories using assays that don't differentiate between variants altering a particular amino acid site should consider whether these are appropriate for use as it is becoming increasingly important to differentiate these variants/amino acid changes for existing and emerging targeted drug therapy treatments.
- Some laboratories provided genomic references instead of cDNA reference sequences. A cDNA reference sequence (NM_XXXXXX.X) is required to interpret the position of the variant according to the HGVS nomenclature (0.2 deduction for not providing a reference sequence).
- HGVS guidelines (21.0.4) state both a genomic reference sequence and cDNA reference sequence are required if the variant extends beyond the transcript, for example for intronic splice variants.
- Laboratories should always include the results for all genes tested (within the scope of the EQA scheme) and not omit the results for genes where no variant was identified. Alternatively, a statement "No other clinically actionable variants were detected" should be included if a variant has been detected.

Case 1

Genotyping

- The mean genotyping score was 1.87 and full marks were achieved by 70.0% (210/300) of laboratories.
- This was a patient tumour biopsy sample, and the genotyping performance was inconsistent during validation, particularly for Block ID 01.22606. This case was therefore marked educationally, with no associated poor performance.
- 82.3% (177/215) laboratories reported that the test failed due to poor sample quality for Block ID 01.22606, the majority requested a new sample for testing or suggested liquid biopsy testing. The remaining laboratories reported results. Eleven laboratories reported variants that were not expected in this sample and not likely to be genuine. Due to the poor sample quality no deductions were given but **we strongly advise that these laboratories review their criteria for acceptance of quality for a DNA sample, and if the quality is poor, results are not reported, and a new sample is requested**. The following results were reported:
    - 177/215 (82.3%) Test failed
    - 19/215 (8.8%) No variant detected
    - 19/215 (8.8%) Variant detected
        - 8/215 (3.6%) *EGFR* NM_005228.5:c.2573T>G p.(Leu858Arg) (Expected result)
        - 3/215 (1.4%) *BRAF* NM_004333.6:c.1799T>A p.(Val600Glu)
        - 3/215 (1.4%) *KRAS* NM_004985.5:c.34G>T p.(Gly12Cys)
        - 1/215 (0.5%) *EGFR* NM_005228.5:c.2155G>A p.(Gly719Ser)
        - 1/215 (0.5%) *KRAS* NM_004985.5: c.34G>A p.(Gly12Ser)
        - 1/215 (0.5%) Deletion in *EGFR* (NM_005228.5) 'exon 19'
        - 1/215 (0.5%) *KRAS* NM_004985.5:c.38G>A p.(Gly13Asp)
        - 1/215 (0.5%) *BRAF* NM_004333.6:c.1790T>C, p.(Leu597Pro)
- Only 5% of laboratories (5/93) reported that testing failed for Block ID 01.29378. Most laboratories reported the expected variant, *EGFR*: NM_005228.5:c.2573T>G p.(Leu858Arg).
- **If testing fails, a full clinical report should be provided, as it would in a clinical setting.**

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Interpretation

- The mean interpretation score was 1.82 and 69.3% of laboratories achieved full marks (165/238) compared to 46% in 2023, a significant improvement. There were no critical interpretation errors this year.
- Some laboratories expressed doubt in the robustness of their result but still reported and interpreted the findings. Results should not be reported if there is doubt in the result and/or the assay has not passed quality metrics as this could be misleading. Instead, a new sample should be requested for testing.
- Some laboratories still provide a generic interpretation that is the same regardless of the genotype obtained. These reports can be easily mis-read and could therefore result in inappropriate treatment of the patient, e.g., it is misleading to discuss resistance to treatment as a general comment when the ACTUAL result indicates sensitivity to therapy. **Clinical interpretations must be tailored to the individual referral reason, the patient tested, and the specific results obtained.**
- Unless it is required by local / national guidance, it is recommended to refer to the class of drugs rather than specific drugs, as treatment choice is a clinical decision based on many factors and more than one therapy option may be available.
- Some laboratories provided biological interpretation without clinical (therapeutic) recommendations in response to the referral. We remind laboratories that supporting information should be uploaded with reports explaining why clinical interpretation has not been provided - for example due to national guidelines – to avoid deduction of marks.
- There was an improvement in the number of laboratories including sensitivity and specificity on their reports, but some laboratories did not report this and only provided the limit of detection (LOD). There are guidelines available for determining these metrics for tests[6,7]
- At EMQN our aim is to educate laboratories about good practice, and we advise that laboratories routinely review (and if necessary, change) their methodology when they miss an actionable variant. With the increase in nucleic acid specific biomarkers for therapy in oncology, it is more important than ever for a laboratory to ensure they are using a test strategy that is able to detect all clinically actionable variants (ISO15189:2022 states "7.3 Examination processes: e) Authorized personnel shall periodically evaluate the examination methods provided by the laboratory to ensure they are clinically appropriate for the requests received").

Clerical Accuracy

- The mean clerical accuracy score was 1.82.
- Many laboratories did not fully describe the reason for referral in the report. It may contain important clinical information and gives context to the reader.
- All results should be presented together on the **first** page of the report and interpreted together, rather than presented as separate results per gene (e.g. *KRAS* results on one page, *EGFR* results on the next page) as this can be confusing for the reader and misleading if for example a page of the report is missed.
- Many laboratories failed to provide patient identifiers on each page of the report, and date of sample receipt, testing and reporting were missing.
- A few reports of excessive length were observed (greater than 10 pages) that appear to be generated from automated reporting systems and included raw data (e.g. graphs for Real-time PCR assays) unnecessarily in reports. Clinical reports should be concise and not contain raw data, please see best practice guidelines for clinical reporting of molecular diagnostic results[2].

Case 2

Genotyping

- The mean genotyping score was 1.97 and 92.8% (285/307) of participants achieved full marks.

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- There were no actionable variants in this sample and there were two critical errors (2/307, 0.70%) due to false positive results, one of which was likely due to a sample transposition (See Table 8).

Case 3

Genotyping

- The mean genotyping score was 1.91 and 87.0% (266/306) of participants achieved full marks.
- There were eight critical genotyping errors (8/306, 2.61%) (See Table 8).
- The *EGFR* variant was mis-called in a number of cases. Bioinformatic pipelines can struggle with deletions and we strongly encourage checking end-points of deletions in a sequence viewer e.g. IGV.
- Some laboratories could not characterise the variant and described it as an 'exon 19 deletion or 'Exon 19 del'. We recommend variants are reported as 'a deletion in exon 19 of the *EGFR* gene', so as to differentiate from a whole exon deletion.
- Some laboratories included the deleted nucleotides in the variant nomenclature. HGVS nomenclature (v21.04) recommends not to describe the deleted nucleotide sequence as the description is longer and contains redundant information.

Case 4

Genotyping

- The mean genotyping score was 1.89 and 82.1% (252/307) of participants achieved full marks.
- There were six critical genotyping errors (6/307, 1.95%) (See Table 8).
- Some laboratories described two individual variants instead of *BRAF* NM_004333.6:c.1798_1799delinsAA. This variant should be described as a delins, not as a substitution, according to HGVS nomenclature prioritisation rules.
- It is not necessary to include the deleted nucleotides in the variant description.

Case 5

Genotyping

- The mean genotyping score was 1.93 and 83.0% (256/308) of participants achieved full marks.
- There were two critical genotyping errors (2/308, 0.65%), both false negative results (see Table 8).
- Laboratories that cannot distinguish between variants in *KRAS* codon 12 are strongly encouraged to change to testing methods that do so, given that there are therapeutic agents available in this clinical context e.g. for the *KRAS* p.(Gly12Cys) variant.

Professional standards

Laboratories are assessed against the guidelines and relevant peer reviewed literature currently available references[2,3]. Other guidelines against which laboratory reports are assessed may include the international nomenclature HGVS[4] and ISO standards (ISO15189)[5].

Assessment team

The assessment of participants' submissions was undertaken by a team of independent, expert assessors.

Table 1: Assessment Team

Assessors	Location	Role
Beatriz Bellosillo	Spain	Assessor
Riziero Esposito Abate	Italy	Assessor

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Ferenc Fazakas	Romania	Assessor
Francesca Fenizia	Italy	Assessor
Stefano Forte	Italy	Assessor
Andrea Gomez Corredor	Canada	Assessor
Val Hyland	Australia	Assessor
Urszula Lechowicz	Poland	Assessor
Lavanya Nambaru	India	Assessor
Hada Navas Fernández	Spain	Assessor
Marta Pereira	UK	Assessor
Giuseppe Perrone	Italy	Assessor
Min Ru Qiu	Australia	Assessor
Pauline Rehal	UK	Assessor
Daniela Righi	Italy	Assessor
Patricia Ruiz Ontanon	Spain	Assessor
Tracy Stockley	Canada	Assessor
Simon Tobi	UK	Assessor
Stefania Tommasi	Italy	Assessor
Pascal Vannuffel	Belgium	Assessor
Bartosz Wasag	Poland	Assessor
Marzena Wojtaszewska	Poland	Assessor

Amendments to the 2024 report following the appeals process

The pre-appeals Lung Cancer (NSCLC) [Tissue] Common Biomarkers cancer Summary Scheme Report v1 was published on the 19th November 2024. There were 32 appeals made by twenty laboratories submitted against the marking of the scheme results. These appeals were reviewed by the members of the scheme assessment team alongside the EMQN team. Eleven of these appeals were rejected, eighteen were upheld and three were partially upheld. Changes to the original marking were made, where applicable, and the ILR's of all laboratories that appealed were updated with the EMQN response. Where relevant, tables and text within this report have also been amended to reflect any updates to marking.

Confidentiality

Details of our confidentiality policies can be found here: https://www.emqn.org/terms-conditions/ in section 4.6 Performance evaluation.

Subcontracted activities

Your EQA provider does not subcontract activities such as EQA planning, evaluation of performance or the authorization of reports. However, some activities are subcontracted, for example the preparation of materials may be performed by suitably accredited providers. Validation of EQA materials and technical advice for setting case scenarios and assessment of results is provided by the EQA team and expert centres.

If your laboratory has sub-contracted part of the analytical process to another organisation / third party, this should be clearly stated on your clinical reports (ISO15189 REQ 6.8.2 and REQ 7.4.1.7)[3].

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Final comments

The assessment team would like to thank all participants for their hard work, prompt return of results and their co-operation during this exercise.

The purpose of the EQA service is to educate and facilitate the raising of standards. Assessors volunteer considerable time and effort to mark the submissions and to provide assistance to laboratories that may require improvement.

We look forward to your participation in the 2025 EQA, and you will be notified by email when registration is available on the EMQN CIC website.

Thank you for participating in this EQA scheme and we hope you have found it a useful EQA exercise.

Kind regards,

Melanie CHEETHAM (MSc.)

Scheme Organiser

tel: +44 (0) 161 757 1591 **web:** www.emqn.org **mail:** office@emqn.org

APPENDICES

Rationale for clinical cases

Case 1

Routine molecular referral with an *EGFR* NM_005228.5:c.2573T>G p.(Leu858Arg) variant present. No clinically actionable variants in the *KRAS* or *BRAF* genes. This case was included to determine how participants would interpret it in the context of EGFR TKI therapy.

On August 19, 2024, after the EQA scheme had been distributed to participants, the Food and Drug Administration approved lazertinib (Lazcluze, Janssen Biotech, Inc.) in combination with amivantamab-vmjw (Rybrevant, Janssen Biotech, Inc.) for the first-line treatment of locally advanced or metastatic non-small cell lung cancer (NSCLC) with deletions in exon 19 of EGFR or *EGFR* p.(Leu858Arg) substitution mutations, as detected by an FDA-approved test. The implementation of this approval was not considered during the assessment this year.

Participation

Table 2: Participation data

Participation Details	Number
Number of registrations	342
Number of withdrawals	17
Number of laboratories that did not submit results	14
Total number of participating laboratories	311

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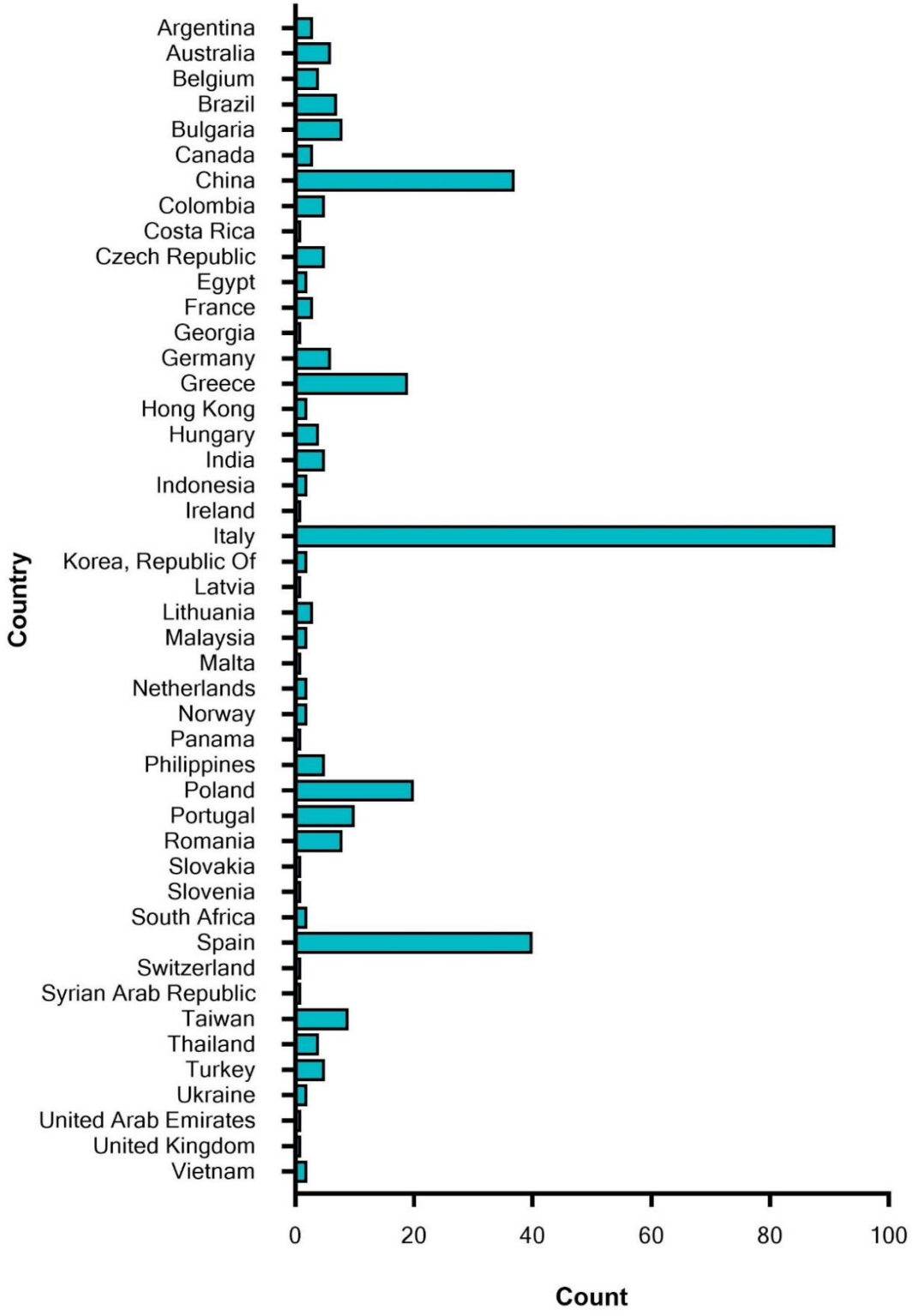
Figure 1: Participating countries



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Samples Provided and Validated Results

The participants received five samples; case 1 derived from a patient tumour biopsy and cases 2-5 manufactured commercially by Horizon Discovery BioSciences Ltd., UK, comprising FFPE scrolls prepared from engineered lymphoblastoid cell lines, some harbouring relevant clinically actionable variants at prescribed allele frequencies. Sections throughout the tissue block for EQA sample 1 were genotyped independently by two external validated laboratories using targeted NGS[¥]. The genotype of EQA samples 2-5 was validated by the manufacturer using ddPCR and by one external validated laboratory using targeted NGS[∞].

Diagnostic requests for the mock clinical cases were sent together with the samples. The expected results are shown in below in Table 3.

Table 3: EQA Sample details and validated results

Case	Name	Sex	Date of Birth (dob)	Referral Reasons	Validated Result[1]
1	Betty KANE	Female	17/06/1972	Betty, a long-time smoker has presented with a cough for several weeks, and shortness of breath during physical exertion. She was admitted to the hospital. Imaging showed a mass of a diameter of approximately 32 mm on the left upper lobe and enlarged bronchial lymph nodes. A core needle biopsy was performed which showed TTF1 positivity. The patient has been diagnosed with lung adenocarcinoma. Material from the biopsy has been sent for molecular diagnostics to evaluate treatment with targeted therapy.	*EGFR* NM_005228.5:c.2573T>G p.(Leu858Arg)[2]
2	Sylvie PASCAL	Female	09/09/1966	Genotype only, no clinical report required.	No clinically actionable variants detected[3] in *EGFR*, *KRAS* or *BRAF*
3	Sam BURBECK	Female	05/11/1969	Genotype only, no clinical report required.	*EGFR* NM_005228.5:c.2236_2253del p.(Glu746_Thr751del) (5% VAF)
4	Hilary ROE	Female	30/08/1970	Genotype only, no clinical report required.	*BRAF* NM_004333.6:c.1798_1799delinsAA p.(Val600Lys) (11% VAF)
5	Allison GREENE	Female	28/03/1970	Genotype only, no clinical report required.	*KRAS* NM_004985.5:c.34G>C p.(Gly12Arg) (10% VAF)

[1]. HGVS has recently updated guidance (v21.0.4) on preferred reference sequences to recommend MANE Select and MANE Plus Clinical.

[2]. The performance of this sample, particularly Block ID 01.22606, was inconsistent during validation so it has been assigned as an educational sample (no poor performance is associated with this sample).
[3]. 'No clinically actionable variants detected' means no clinically relevant variants were detected within the regions tested.

¥ 1) Single-primed PCR enrichment using a QIAseq Targeted DNA custom panel with Unique Molecular Identifiers (UMIs) and Illumina Next Generation Sequencing on NovaSeq6000. 2) Library prep & hybridisation with NGS panel: GALEAS Tumor (Nonacus). Sequencing: Illumina NextSeq2000 P3, 300 cycles.

∞ For mutation analysis, targeted NGS (Thermo Fisher Oncomine Dx derived amplicons - library preparation with Ion AmpliSeq Library kit 2.0 - Sequencing on Ion Torrent PGM platform / Torrent Suite 5.12.3 - Variant Caller with 5.12.0.4 under "Generic - PGM (3xx) - Somatic - Low Stringency" settings - Annotation with Ion Reporter software 5.20.2.0). Genes tested are indicated in uploaded Excel file. Targeted coverage =1000 reads for all amplicons.

For mutation analysis, the tested genes are not sequenced entirely (only targeted gene exons or parts of them are investigated). The methodology will not detect large deletions/insertions or inversions. The minimum detectable mutant allele ratio is approximately 5%. All results provided are according to annotation of the Ion Reporter software 5.20.2.0.

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Evaluation criteria of the reports

The assessment assigned marks to the genotyping accuracy and the interpretation of the results the laboratories provided in their reports. Patient details and clerical accuracy were also assessed. The full score for each category was 2.00. The assessors considered the accuracy, clarity and clinical relevance of the report issued to the referring clinician, with reference to available professional standards and publications [2,3].

Table 4: EQA Marking Criteria

Category	Category	Criterion	Deduction
All Cases	Genotyping	• Reporting variants in genes that are outside the scope of the Lung (NSCLC) cancer (Tissue) COMMON BIOMARKERS EQA scheme	0.2
		• Reference sequence is missing / incorrect / inconsistent	0.2
		• Reference sequence version / transcript number is missing / incorrect / inconsistent	0
		• LRG reference sequences are no longer generated or updated. We recommend you change to MANE Select or MANE Plus Clinical	0
		• Comment only	0
		• Comment with deduction	0.2
		• Comment with deduction	0.5
		• Comment with deduction	1
		• Not marked	0
		• Withdrawn from scheme	0
Case 1	Genotyping	• Not correctly using HGVS nomenclature (for either nucleotide or protein)	0.2
		• No deduction	0
		• Doubtful results should not be reported (e.g. report states that sample was of sub-optimal quality for testing)	1
		• Failure to report results for all mandatory genes (*EGFR*, *KRAS* and *BRAF*) with no explanation provided	0.2
		• Block ID 01.22606 or 01.29378: Test failure due to low cellularity of sample / poor sample quality, giving no result for the sample and stated that a repeat sample should be requested	0
		• Block ID 01.22606 or 01.29378: Test failure due to low cellularity of sample / poor sample quality, giving no result for the sample and did not state that a repeat sample should be requested	0.5
	Interpretation	• All essential interpretative elements provided	0
		• Critical interpretation error	2
		• "No clinical interpretation given (with no explanation provided)"	1.5
		• Limited clinical interpretation e.g. insufficient clinical information is provided to inform clinical decision-making	1
		• Misleading interpretive comment / generic interpretation given irrespective of the genotype	1
		• Interpretation given but no patient-specific comment related to licensed drug therapy	0.5
		• Interpretation of variants that are not clinically relevant to the clinical question / are outside the scope of this EQA scheme	0.2
		• Clerical error(s) causing potential for patient harm e.g. incorrect or inconsistent use of patient name in the body of the report	0.5
		• No statement about the assay /testing method used	0.5
		• Failure to provide an adequate analytical scope of the test(s) used i.e. which exons / codons / variants are covered and which types of variants are not covered	0.2
		• Failure to provide adequate details of test performed (for example, limitations, LOD, accuracy, sensitivity and specificity) in relation to the suitability of the material provided	0.2

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		• Insufficient information provided on the NGS testing methodology: strategy (i.e. WGS, WES, targeted), depth, coverage, chemistry, platform etc.	0.2
		• Comment only	0
		• Comment with deduction	0.5
		• Comment with deduction	1
		• Comment with deduction	1.5
		• Not marked	0
		• "Not marked (due to critical genotyping error)"	0
		• Withdrawn from scheme	0
	Clerical Accuracy	• All essential patient identifiers present and no significant clerical errors	0
		• No restatement of the reason for patient referral	0.2
		• DOB incorrect or missing	1
		• Patient name has a spelling error	0.5
		• Patient gender is not specified on the report. Whilst not essential, this is another additional identifier of the patient, and we recommend its inclusion on your report	0
		• Failure to provide patient identifiers on each page of the report	0.2
		• Failure to provide the dates of sample receipt / testing or reporting	0.2
		• Failure to provide the sample type	0.2
		• The sample type provided is incorrect	0.2
		• No neoplastic cell content reference on report	0.5
		• No block number provided	0.2
		• No section ID provided	0.2
		• There is no evidence that the report was authorised i.e. report not signed	0
		• Incorrect pagination (use if states Page 2 of 1, for example)	0.2
		• Failure to provide correct pagination e.g. pagination missing or only states Page 1 instead of Page 1 of 1 etc.	0.2
		• Failure to provide a clear presentation of results	0
		• Failure to anonymise report	0
		• The essential clinically relevant information is 'lost' in this long report. Consideration should be given to reducing the length of the reports	0
		• The essential clinically relevant information is 'lost' in this long and overly complicated report. There are too many unnecessary tables and figures. Consideration should be given to simplifying and reducing the length of the reports	0
		• Comment only	0
		• Comment with deduction	0.2
		• Clear and concise report	0
		• Not marked	0
		• Not marked (due to critical genotyping error)	0
		• Withdrawn from scheme	0
Case 2, 3, 4 & 5	Genotyping	• Correct result reported	0
		• Critical genotyping error	2
		• Failure to test all mandatory genes (*EGFR*, *KRAS* and *BRAF*) with no explanation provided	0.2
		• Reporting variants in the *EGFR*, *KRAS* or *BRAF* genes that are not clinically relevant to the question asked in this case	0.2
		• Test failure giving no result for the sample and stated that a repeat sample should be requested	0
		• Test failure giving no result for the sample and did not state that a repeat sample should be requested	0.5
	Genotyping	• Genotype mis-positioned or mis-called (e.g. incorrect base/amino acid detected)	1

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Cases 1, 3, 4 & 5		• Variant only reported at the protein level. As this is a DNA based test it should also be reported at the nucleic acid level	0.2
		• Minor HGVS error e.g. missing brackets around the protein or p. inside brackets	0
Case 3	Genotyping	• Correct result within the limitations of the testing performed e.g. 'deletion in exon 19 of the *EGFR* gene'	0
		• Not correctly using HGVS nomenclature (for either nucleotide or protein) e.g. inclusion of deleted bases	0
		• Use of 'Ex19del' or '19del' etc. could be misleading as it may be interpreted as a deletion of *EGFR* exon 19 rather than a deletion in EGFR exon 19. Better to say 'A deletion in exon 19 of the *EGFR* gene' if your test cannot distinguish the exact variant.	0
Case 4	Genotyping	• Correct result within the limitations of the testing performed e.g. test cannot distinguish this variant from others at *BRAF* codon 600	0
		• Not correctly using HGVS nomenclature (for either nucleotide or protein) e.g. *BRAF* NM_004333.6:c.1798_1799delGTinsAA p.(Val600Lys) instead of *BRAF* NM_004333.6:c.1798_1799delinsAA p.(Val600Lys)	0
Case 5	Genotyping	• Correct result within the limitations of the testing performed i.e. test cannot distinguish this variant from others at *KRAS* codon 12. We strongly recommend that you consider a testing strategy that can uniquely identify the different variants at *KRAS* codon 12 or consider sending this sample to another laboratory which has a more comprehensive / complimentary testing strategy as there are licensed therapies available for a subset of Lung (NSCLC) cancer patients that harbour a *KRAS* NM_004985.5:c.34G>T p.(Gly12Cys) variant.	0
		• Not correctly using HGVS nomenclature (for either nucleotide or protein) e.g. G12X	0.2

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Results: summary statistics

The mean scores for genotyping/analytical, interpretation, clerical accuracy and the total mean score for all participating laboratories are given below in Table 5. A summary of the number of critical errors per case is provided in Tables 6 & 7.

Table 5: Mean Scores

Category		Case 1	Case 2	Case 3	Case 4	Case 5
Genotyping	Mean (SD)	1.87 (0.24)	1.97 (0.18)	1.91 (0.35)	1.89 (0.34)	1.93 (0.24)
	Median (SD)	2.0 (0.24)	2.0 (0.18)	2.0 (0.35)	2.0 (0.34)	2.0 (0.24)
Interpretation	Mean (SD)	1.82 (0.37)	n/a	n/a	n/a	n/a
	Median (SD)	2.0 (0.37)	n/a	n/a	n/a	n/a
Patient Identifiers & Clerical Accuracy	Mean (SD)	1.82 (0.37)	n/a	n/a	n/a	n/a
	Median (SD)	2.0 (0.37)	n/a	n/a	n/a	n/a

There were 13 laboratories (13/311, 4.2%) that made critical genotyping errors for this EQA (see Table 6). Ten laboratories reported one error, two laboratories reported errors in two cases, and one laboratory reported errors in four cases (probably due to sample transpositions).

Table 6: Critical Genotyping Errors

Category	Case 1	Case 2	Case 3	Case 4	Case 5	Total
Number of cases completed	300	307	306	307	308	1528
Number of laboratories with full marks	210	285	266	252	256	1269
Number of critical errors	0	2	8	6	2	18
Error rate (%)	0	0.65	2.61	1.95	0.65	1.18

Table 7: Critical Interpretation Errors

Category	Case 1	Case 2	Case 3	Case 4	Case 5	Total
Number of cases completed	238	n/a	n/a	n/a	n/a	238
Number of laboratories with full marks	165	n/a	n/a	n/a	n/a	165
Number of critical errors	0	n/a	n/a	n/a	n/a	0
Error rate (%)	0	n/a	n/a	n/a	n/a	0

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Results: Critical genotyping Errors Summary

Table 8 below shows a breakdown of the critical genotyping errors made by laboratories that participated in this EQA scheme.

Table 8: Summary of critical genotyping errors made in this EQA scheme

Case	Error	Description	Number of laboratories
2	False positive	Incorrectly reported *KRAS* NM_004985.5:c.34G>C p.(Gly12Arg) Possible sample transposition with case 5	1
2	False positive	Incorrectly reported *BRAF* NM_004333.6:c.1798_1799delinsAG p.(Val600Arg) Using BRAF Codon 600 Mutation Analysis KIT II (EntroGen)	1
3	False negative	Failed to report *EGFR* NM_005228.5:c.2236_2253del p.(Glu746_Thr751del) at 5% VAF Using ACTION ONCO KIT DX Ç8AUTOMATIC	1
3	Variant misclassification	*EGFR* NM_005228.5:c.2236_2253del p.(Glu746_Thr751del) at 5% VAF reported as a VUS	2
3	False positive and false negative	Incorrectly reported *BRAF* 'V600K/V600R/V600M; p.(Val600Lys)/p.(Val600Arg)/p.Val600Met); c.1798_1799GT>AA/c.1798_1799GT>AG/c.1798G >A' Possible sample transposition with case 4	1
3	False negative	Failed to report *EGFR* NM_005228.5:c.2236_2253del p.(Glu746_Thr751del) at 5% VAF Using SureSelect XT HS (Agilent)	1
3	False positive and false negative	Incorrectly reported *EGFR* NM_005228.5 c.2573T>G p.(Leu858Arg) Using cobas® EGFR Mutation Test v2 (Roche)	1
3	False positive and false negative	Incorrectly reported *EGFR* NM_005228.5: c.2573T>G p.(Leu858Arg) Using EGFR Mutation Analysis Kit (EntroGen)	1
3	False positive and false negative	Incorrectly reported ***BRAF*** NM_005228.5:c.2236_2253del p.(Glu746_Thr751del) Reported variant in wrong gene	1
4	False negative	Failed to report *BRAF* NM_004333.6:c.1798_1799delinsAA p.(Val600Lys) Using Oncomine Precision Genexus (GX) (Thermo Fisher)	1

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4	False negative	Failed to report *BRAF* NM_004333.6:c.1798_1799delinsAA p.(Val600Lys) Using cobas 4800 BRAF V600 Mutation Test (Roche)	1
4	False negative	Failed to report *BRAF* NM_004333.6:c.1798_1799delinsAA p.(Val600Lys) Using Oncomine Comprehensive Assay v3 (Thermo Fisher)	1
4	False positive and false negative	Incorrectly reported Exon 19 deletion in *EGFR* Possible sample transposition with case 3	1
4	False positive and false negative	Incorrectly reported *EGFR* NM_005228.5: c.2573T>G p.(Leu858Arg) Using Oncomine Focus Assay (Thermo Fisher)	1
4	False positive	Incorrectly reported *KRAS* 'NM_033360.2 p.G12A' Using Oncology 59-Gene Variant Assay (Geneplus)	1
5	False negative	Failed to report *KRAS* NM_004985.5:c.34G>C p.(Gly12Arg) (10%) Using KAPA HyperExome Plus Kit (Roche)	1
5	False negative	Failed to report *KRAS* NM_004985.5:c.34G>C p.(Gly12Arg) (10%) Possible sample transposition with case 2	1
TOTAL			**18**

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Results: Methodology used

Figure 2.

Commercial kit names as provided by participants

Methodology	
NGS Targeted	**192**
3DMed Diagnostics	**1**
3DMed Onco Core™ Tissue Kit	1
ABclonal	**1**
Rapid Plus DNA Lib Prep Kit For Illumina	1
Agilent	**7**
Magnis SureSelect XT HS DNA Reagent kit	1
SureSelect XT HS	4
SureSelect XT Custom Enrichment	1
SureSelect Cancer CGP Assay	1
AmoyDx®	**3**
Essential NGS Panel	1
HANDLE Classic NGS Panel	2
ArcherDX (IDT)	**8**
FusionPlex® Lung v2	5
VariantPlex® Solid Tumor Focus	2
VariantPlex® Solid Tumor	1
BioVendor	**2**
FastGEN Kit	2
BOKEbio	**1**
96 rxn TargetCap® Hybridization and Wash Kit	1
Diatech Pharmacogenetics	**41**
Myriapod® NGS Cancer Panel DNA	41
EntroGen	**1**
NGS Targeted Hotspot Panel	1
GenePlus	**2**
Oncology Multi Gene Variant Assay	1
Oncology 59 Gene Variant Assay	1
Health in Code	**2**
Action OncoKitDx (Automatic)	2
Illumina	**11**
AmpliSeq™ Focus Panel	7
Trusight™ Oncology 500	1
Trusight™ Tumor 15	3
Integrated DNA Technologies (IDT)	**2**
xGen™	1
rhAmpSeq™ CRISPR Library Kit	1
MGI	**5**
MGIEasy Universal DNA Library Prep Set v1.0	1
Target Area Capture Universal Reagent	2
NanOnco Plus Panel v3.0	1
Qiagen	**4**
Qiaseq DNA Human Actionable Solid Tumour Panel	1
Qiaseq Targeted DNA Human Lung Cancer Panel	1
Qiaseq Targeted DNA Custom Panel	2
Revvity	**1**
NEXTflex® DNA-Seq Kit	1
Roche	**3**
AVENIO Tumor Tissue Expanded Kit v2	2
KAPA Hyperchoice	1
SOPHiA	**3**
SOPHiA Solid Tumor Solution	3
Thermo Fisher Scientific	**92**
Ion Ampliseq™ Colon and Lung Cancer Research Panel	4
Ion Ampliseq™ Cancer Hotspot Panel v2	6
Ion Ampliseq™ Custom Targeted NGS Testing Panel	6
Ion Ampliseq™ Library Kit	8

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Oncomine™ Comprehensive Assay v3	5
Oncomine™ Focus Assay	15
Oncomine™ Precision Assay	26
Oncomine™ Precision Assay GX (Genexus)	18
Oncomine™ Solid Tumour DNA Kit	1
Oncomine™ Dx Target Test	1
Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay	1
Ion Torrent Oncomine™ Dx Express Test	1
Twist	**2**
Twist Library Preparation Kit	1
Twist Fast Hybridization and Wash Kit	1
Other	**2**
Oncology Multi Gene Mutations Detection Kit	2
In House Design	**10**
NGS Whole Exome	**1**
Roche	**1**
KAPA HyperExome Plus Kit	1
Real-Time/ Fluorescent PCR	**120**
ACCB	**2**
Gene Mutations Detection Kit (Fluorescent PCR) EGFR, KRAS, BRAF	2
AmoyDx®	**51**
5 Gene Mutations Detection Kit	4
EGFR 29 Mutations Detection Kit	11
Human EGFR Mutations Detection Kit	11
BRAF V600 Mutation Detection Kit	7
KRAS Mutation Detection Kit	10
Pan Lung Cancer PCR Panel	5
KRAS/NRAS/BRAF Mutations Detection Kit	1
Human BRAF V600E Mutations Detection Kit	2
Biocartis	**36**
Idylla™ EGFR Mutation Test (CE-IVD)	21
Idylla™ KRAS Mutation Test (CE-IVD)	8
Idylla™ NRAS-BRAF Mutation Test (CE-IVD)	3
Idylla™ BRAF Mutation Test (CE-IVD)	4
Cellomics (Shenzhen) Co., Ltd	**1**
Human EGFR Gene Mutation Detection Kit	1
Diatech Pharmacogenetics	**52**
EasyPGX® ready EGFR	20
EasyPGX® ready KRAS	15
EasyPGX® ready BRAF	16
EasyPGX® ready EGFR plus	1
EntroGen	**7**
Entrogen Colorectal Cancer Mutation Detection Panel	1
Entrogen RAS mutation analysis kit v2.2	1
EGFR Mutation Analysis Kit	3
EntroGen KRAS Mutation Analysis Kit	1
EntroGen BRAF Mutation Analysis Kit	1
PanaGene	**1**
PANAMutyper™ R	1
Pentabase	**8**
SensiScreen FFPE EGFR qPCR Assay Exon 18+19+20+21 Multiplex (E1)	3
SensiScreen FFPE KRAS qPCR Assay Exon 2 Simplex 1 (K5)	2
SensiScreen FFPE BRAF qPCR Assay V600 Simplex (B2)	3
Qiagen	**1**
therascreen® EGFR RGQ PCR Kit version 2	1
Roche	**42**
cobas® EGFR Mutation Test v2	25
cobas® 4800 BRAF V600 Mutation Test	2
BRAF/NRAS Mutation Test (LSR)	7
KRAS Mutation Test v2 (LSR)	8
Surplex	**1**

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Surplex® EGFR Mutation Kit, Surplex® BRAF Mutation Kit, Surplex® KRAS Mutation Kit	1
Thermo Fisher	**1**
Thermo Fisher TaqMan™ Mutation Detection Assay.	1
High Resolution Melting	**1**
In House Method	**1**
Pyrosequencing	**1**
Qiagen	**1**

Not stated	1
Sanger Sequencing	**1**
Thermo Fisher	**1**
Applied Biosystems™ Sanger Sequencing Kit	1
Other	**1**
ViennaLab	**1**
EGFR XL StripAssay® and KRAS XL StripAssay®	1

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Amendments to this summary EQA report

Version	Page	Section	Change	Published
1	-	-	None	27/01/2025
2				
3				

Authorisation

This document has been authorised / approved on behalf of EMQN CIC by:

Dr. Simon Patton on 27th January 2025

CEO

tel: +44 (0) 161 757 1591 **web:** www.emqn.org **mail:** office@emqn.org