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Pilot EQA for Multibiomarker Testing in Cell Free DNA (cfDNA)

Contents

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20th December 2024

Dear Colleague,

Thank you for participating in this pilot external quality assessment (EQA) to assess cell-free DNA (cfDNA) testing for multiple tumour biomarkers. This EQA has been provided as an IQN Path collaboration between six External Quality Assessment (EQA) providers (AIOM, CBQA, EMQN, GenQA, Gen&Tiss, and RCPAQAP). The data analysis and preparation of this report was undertaken by GenQA.

The harmonised marking has been completed for all EQA providers and your individual laboratory scores were released to you by your own EQA provider. Each EQA provider has issued their laboratory scores and an EQA Summary Report. This report is an overarching summary which collates the results from all EQA providers and discusses the issues raised by the assessors during the marking process.

Background

Many laboratories are implementing testing of cfDNA to detect the presence of oncogenic somatic variants in plasma samples. As the clinical utility of this testing is recognised, the requirement for the number of targets tested is increasing. In order to help laboratories develop and deliver high quality testing, IQN Path delivered a pilot EQA for testing plasma samples for the detection of multiple targets.

EQA Design and Purpose

This EQA scheme was designed to enable laboratories to test plasma cfDNA for the presence of multiple biomarkers. Participating laboratories were assessed for their ability to undertake variant testing in cfDNA for a range of clinically significant variants involving the following genes: *BRAF, BRCA1, BRCA2, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, IDH1, KIT, KRAS, NRAS, PIK3CA, STK11, ALK, FGFR1, FGFR2, FGFR3, NTRK1, NTRK2, NTRK3, RET* and *ROS1*.

The assessment was twofold:

1. The testing accuracy assessment was carried out for two multiplex cfDNA samples and
2. An evaluation of the standard of clinical reporting against three categories (genotyping, interpretation, and clerical accuracy) was carried out for three cfDNA samples with associated clinical case scenarios, with the objective of helping laboratories to standardise and improve their reporting.

Feedback from the assessment is provided in the form of both individual laboratory reports (ILRs) and this EQA summary report.

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

- Genotype artificial cfDNA samples accurately,
- 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 information and identifiers.

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

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Participation

Sixty laboratories from 17 different countries (*Appendix 1, Figure 4*) registered to participate as follows: AIOM (10), CBQA (7), EMQN (12), GenQA (13), Gen&Tiss (10), and RCPAQAP (8). Four of the EQA providers are national schemes (France, Italy, Canada and Australia) therefore their participating laboratories were from a single country. All laboratories were selected to participate in this pilot EQA based on responses in an expression of interest survey. Inclusion criteria for participating laboratories included accreditation status and level of experience in providing a clinical diagnostic service for variant testing in cfDNA. Of the 60 laboratories selected to participate in this pilot EQA, 50 returned results by the assessment deadline, equating to a participation rate of 83%.

Samples Provided and Testing Required

EQA participants were provided with cfDNA samples for multiple biomarker testing using their routine analytical pipeline(s). cfDNA, purchased from a commercial manufacturer (SensID GmbH, Germany), was used as reference materials for this EQA. Five artificial plasma samples were distributed for testing and all participating laboratories, regardless of which EQA provider they participated through, were supplied with the same samples from the same batch of material. Each sample was supplied with a corresponding patient name, date of birth and testing requested.

Two samples contained multiple biomarkers and laboratories were assessed for genotyping only (Cases 1 and 2). These two samples were artificially manufactured to contain 400ng of cfDNA supplied in 5mL of synthetic plasma.

Three samples were supplied with mock clinical referrals for participants to test using their routine diagnostic pipeline and report using the laboratory's routine format (Cases 3, 4 and 5). These three samples were artificially manufactured to contain 240ng of cfDNA supplied in 3mL of synthetic plasma.

Participants were instructed to report results using an online form hosted on SurveyMonkey and via direct submission of clinical reports to their respective EQA provider.

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Scheme Report on Behalf of the IQN Path cfDNA For Multiple Biomarkers Working Group

All Cases

The five plasma samples provided and the associated validated genotype result are summarised in *Table 1*. Details of sample validation are included in the Appendix (*Appendix 2, Table 6*).

Table 1. Summary of clinical cases and validated results.

Case	Patient details	Referral Reasons	Validated Genotype Result		
			Gene	Reference sequence	HGVS nomenclature (Variant Allele Frequency)
1	Jennifer WEBB 14/08/1959 Female	N/A No clinical case scenario supplied.	*KRAS*	NM_004985.5	c.436G>A p.(Ala146Thr) (0.5%)
			STK11	NM_000455.5	c.842del p.(Pro281fs) (5%)
			PIK3CA	NM_006218.4	c.3140A>G p.(His1047Arg) (2%)
			FGFR3	NM_000142.5	c.1118A>G p.(Tyr373Cys) (5%)
			IDH1	NM_005896.4	c.395G>A p.(Arg132His) (2%)
			BRCA2	NM_000059.4	c.7934del p.(Arg2645fs) (10%)
2	Erika VARGA 22/07/1963 Female	N/A No clinical case scenario supplied.	*EML4::ALK*	*EML4* NM_019063.5 *ALK* NM_004304.5	*EML4* exon 13 *ALK* exon 20
			CD74::ROS1	*CD74* NM_004355.5 *ROS1* NM_002944.3	*CD74* exon 6 *ROS1* exon 34
			TPM3::NTRK1	*TPM3* NM_152263.4 *NTRK1* NM_002529.4	*TPM3* exon 7 *NTRK1* exon 10
			FGFR3::TACC3	*FGFR3* NM_000142.5 *TACC3* NM_006342.3	*FGFR3* exon 17 *TACC3* exon 11
3	Petra ARITI 22/05/1953 Female	Patient has metastatic lung adenocarcinoma and has had first line chemotherapy and is being considered for further treatment.	*EGFR*	NM_005228.5	c.2303_2304insTGTGGCCAG p.(Ala767_Val769dup) (4%)
4	Racquel MOREAU 13/05/1948 Female	Patient was diagnosed with metastatic melanoma and testing of the original sample failed. Testing of liquid biopsy sample is requested to determine treatment options.	*BRAF*	NM_004333.6	c.1799T>A p.(Val600Glu) (1%)
5	Leona REID 15/12/1954 Female	Patient with metastatic lung adenocarcinoma. Testing of the original biopsy found a deletion in exon 19 using a real time PCR based method and the patient received first line first generation EGFR TKI treatment and is now in clear clinical progression. No tissue sample or cytology specimen of progressing disease is available due to their poor clinical condition. Testing of the patient's plasma sample for EGFR gene variants has been requested.	*EGFR*	NM_005228.5	c.2237_2251del p.(Glu746_Thr751delinsAla) (deletion in exon 19) (1%)
					c.2369C>T p.(Thr790Met) (0.5%)

Genotyping

- There were 50 laboratories participating in this pilot EQA for multibiomarker testing in cfDNA. In total, across the five cases, 26 critical genotyping errors were reported by 20 laboratories; the percentage of laboratories reporting critical genotyping errors was 40% (20/50) and the overall error rate was 10.4% (26/250). The mean genotyping score was 1.64 (out of a total possible score of 2.0).
- The nature of cfDNA, where variants may be present at lower variant allele frequencies (VAF) than found in solid tumour samples, requires a higher level of test sensitivity to detect the variants. Therefore, the testing of circulating tumour DNA (ctDNA) in cfDNA requires the use of methods which are more sensitive than those required to test DNA extracted from solid tumour tissue. Consideration should be taken regarding the applicability of a chosen assay and associated limit of detection (LOD) for the testing of cfDNA samples.

- The use of Human Genome Variation Society (HGVS) nomenclature[1] was generally of a high standard. However, several laboratories did receive minor deductions for not using or using incorrect HGVS nomenclature. This included not using c. and p. nomenclature when reporting variants, only providing HGVS protein nomenclature despite using DNA based assays and not including the inserted nucleotides for Case 3.
- According to HGVS guidelines[1] and the HUGO Gene Nomenclature Committee (HGNC) recommendations[2], the designation of gene fusions should use :: to separate the genes in fusions and that the fusions should be described as 5' to 3'. According to HGVS guidelines, use of a hyphen ('-') between gene names denotes a read-through variant. Several laboratories failed to use the correct gene fusion nomenclature for Case 2.
- Six laboratories (6/50, 12%) failed to include or provided an incorrect reference sequence on their report. The reference sequence of reported variants should always be included on the report as it constitutes part of the variant nomenclature. Gene reference sequences should be included in reports even if commercial kits are being used (and do not reference genes within the kit inserts). Even when a laboratory is using a commercially available assay to perform a test, it should be possible to determine the reference sequence for the gene(s) tested and this EQA provided information regarding reference sequences in the distribution letter.

Interpretation

The interpretation category was assessed using a set of pre-defined comments (*Appendix 3, Table 7*), as agreed by the working group.

- Where local/national policy allows, a biological and clinical interpretation of the result in the context of the clinical referral, should always be provided in a diagnostic test report. This enables the report receiver to understand the significance of the variant detected, and how it relates to the clinical presentation in the patient, if at all. If the detected variant(s) is associated with an approved targeted therapy, a statement reflecting this information is acceptable as an interpretive comment.
- Across all cases, five laboratories (5/50, 10%) failed to provide sufficient details on testing methodology. Information such as scope of testing (e.g. genes (including transcript accession numbers) included), analytical sensitivity and specificity, and variant allele frequency cut-offs (i.e. LOD) should be clearly stated on the report to allow the report receiver to make a full and informed interpretation of the result, in the context of the testing performed. In addition, there should be sufficient information regarding the NGS testing methodology, including the platform used and/or manufacturer and/or strategy (i.e. whole exome sequencing, targeted), depth and coverage.
- In this EQA, where several laboratories did not detect any variants in the sample due to the variants not being within the assay scope or below the LOD, there was a lack of understanding of the limitations of cfDNA testing. When reporting a case with no actionable variants in a cfDNA sample it is recommended to include a statement regarding the reduced sensitivity for detecting variants in a plasma sample, including considering the risk of no circulating tumour DNA being present in the tested sample at the time it was acquired. Therefore, in addition, repeat testing should be recommended for a cfDNA sample with no variants detected or recommend testing of a tumour sample if appropriate. Caution should also be taken to not over-interpret no actionable variants in cfDNA with regards to therapeutic potential for the patient as cfDNA analyses are known to have reduced sensitivity for detecting variants.
- It is not recommended to use specific drug names on reports when multiple drugs targeting the same variants are available.

Patient Identifiers and Clerical Accuracy

During assessment, a series of pre-defined comments agreed by the project team, were applied to the assessment of this category. The standard of clerical accuracy was generally good across all cases. However, there were some recurrent errors which meant the reports did not comply with ISO 15189[3] as follows:

- Either no pagination or just 'page 1' was stated on the reports by a number of participants. It is important to include the pagination and number of pages on the report i.e. page 1 of 2, as this makes it easier to ensure that there are no pages missing from the report.

- Three laboratories did not restate the referral reason in full. This information is required to interpret the molecular genetic testing results in the context of the clinical question.
- Clear and accurate identification of the patient undergoing testing is a crucial element of the reporting process. One participant received a minor deduction for patient identifiers being incorrect in one case, as they provided an incorrect date of birth.
- Several participants did not include sufficient or accurate details of the sample being tested. The date of sampling, sample receipt or date of reporting was missing from the reports of several participants. The date of sampling and receipt is of particular importance for cfDNA samples with respect to the stability of the cfDNA. The type of sample tested (i.e. plasma) was also missing or inaccurate on several reports. Lastly, reports should include the sample identifiers, in this instance the REF and LOT numbers, of the specimen tested as a means of tracking the sample on which testing was performed.
- Several participants did not provide evidence of authorisation on their report.
- Participants are encouraged to review the length of their reports to ensure the essential clinically relevant information is not lost in a long report. Reports can be 1-2 pages and include all the necessary information.
- Several 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.

Case 1

Genotyping

- The mean genotyping score for this case was 1.21 marks (out of 2.0) for the 47 laboratories that analysed this multiplex sample. The results for each gene are summarised in *Table 2* and *Figure 1.*

Table 2. Laboratory results for each variant in Case 1.

Variant	***VAF Range (Average)***	***Number of laboratories***		
		Detected variant	***Did not report variant***	
KRAS **c.436G>A p.(Ala146Thr)**	**Expected 1%** 0.2-1.5% (0.6%)	29 (62%)	Critical error	6 (11%)
			Below LOD	10 (23%)
			Out of scope	2 (5%)
PIK3CA **c.3140A>G p.(His1047Arg)**	**Expected 2%** 1.8-4.4% (2.8%)	43 (91%)	Critical error	1 (2%)
			Below LOD	1 (2%)
			Out of scope	2 (5%)
IDH1 **c.395G>A p.(Arg132His)**	**Expected 2%** 0.7-3.0% (1.9%)	43 (91%)	Critical error	1 (2%)
			Below LOD	1 (2%)
			Out of scope	2 (5%)
FGFR3 **c.1118A>G p.(Tyr373Cys)**	**Expected 5%** 1.3-8.7% (4.4%)	41 (87%)	Critical error	2 (5%)
			Out of scope	4 (9%)
STK11 **c.842del p.(Pro281fs)**	**Expected 5%** 3.3-6.1% (4.9%)	18 (38%)	Critical error	4 (9%)
			Out of scope	25 (53%)
BRCA2 **c.7934del p.(Arg2645fs)**	**Expected 10%** 8.4-14.5% (10.4%)	17 (36%)	Critical error	4 (9%)
			Out of scope	26 (55%)

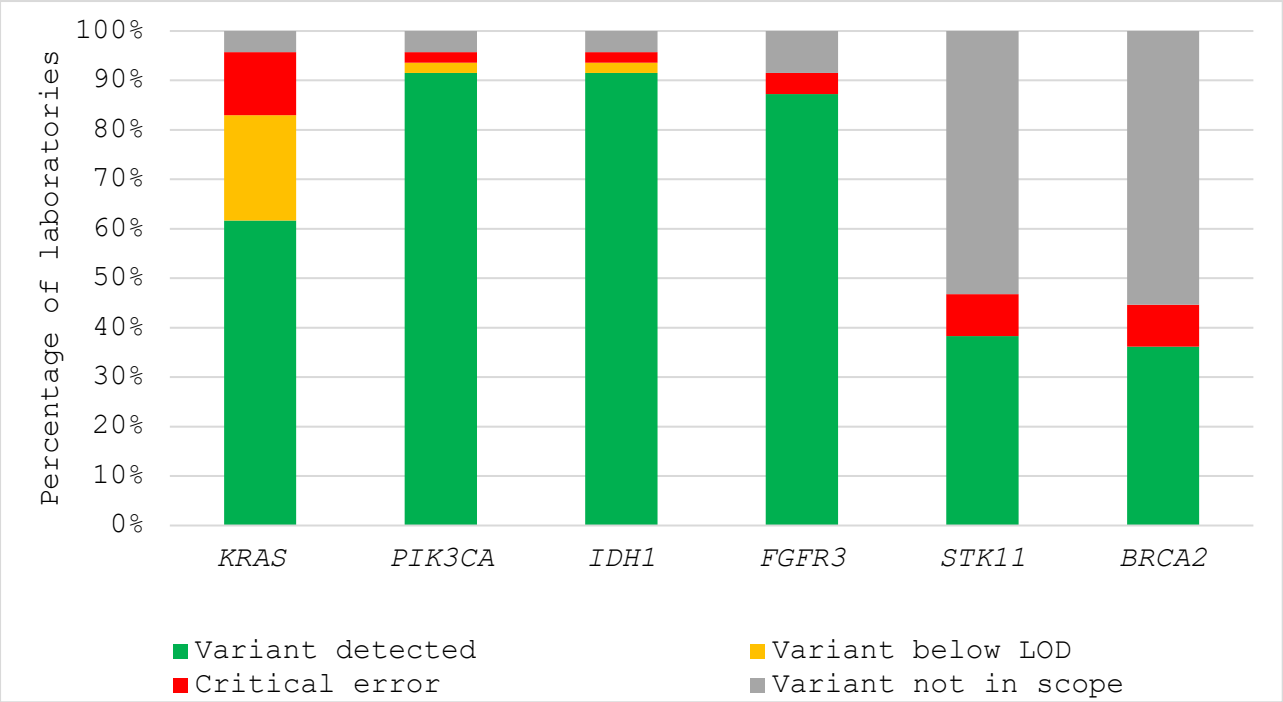

Figure 1. Summary of variant results in Case 1.

- Fourteen laboratories (14/47, 30%) received a critical genotyping error.
  - Twelve laboratories received critical genotyping errors for not reporting one or more of the variants despite the variant being within the scope of the laboratories' assay and above the reported LOD.
  - Two further laboratories received critical genotyping errors for a false positive result. Both laboratories reported a *BRAF* c.1798G>A p.(Val600Met) at 0.1% or 0.4%. This variant was not reported by any of the validating laboratories or by any other laboratories.
- The *KRAS* c.436G>A p.(Ala146Thr) variant was present at an average variant allele frequency (VAF) of 0.6%. Ten laboratories did not report this variant, however where the laboratories' stated LOD was 0.5% or above they were marked as correct within the limitations of the assay. This is a good example of the importance of including the limitations of the assay being utilised e.g. LOD, on a clinical report.
- There was a wide range of genes included in laboratories scope of assay. This is summarised in *Figure 2.*
- Twenty-three out of 47 participating laboratories (49%) received full marks.

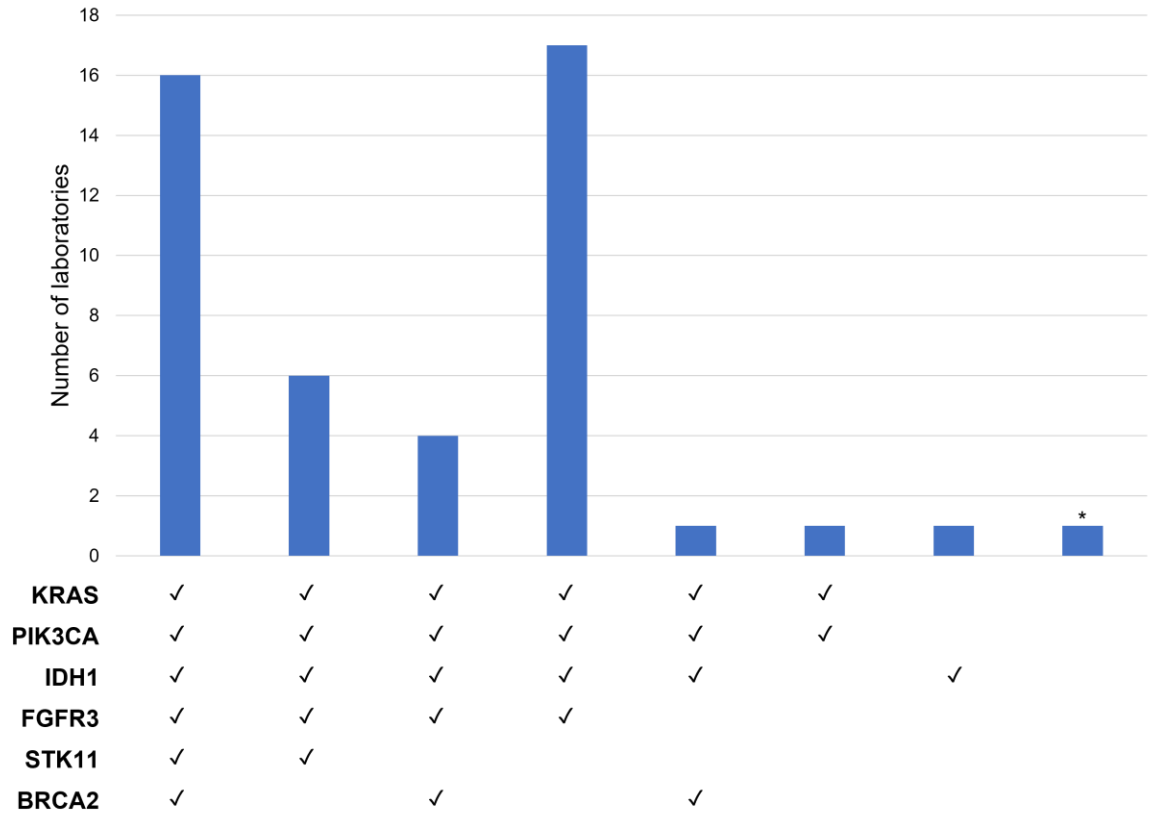

*Figure 2. Genes included in the scope of laboratories' assay. *One laboratory analysed the sample but their scope did not include any of the six genes.*

Case 2

Genotyping

- The mean genotyping score was 1.48 for this case.
- Sixteen laboratories were not marked or did not participate in this case for varying reasons;
  - Four laboratories did not have the fusion genes requested included in their scope
  - Seven laboratories only test fusions by RNA. This artificial sample contains cfDNA only so would not be suitable for an RNA-based assay
  - One laboratory failed the sample
  - Four laboratories did not provide a report for this case and did not provide a reason.
- The results for each fusion are summarised in *Table 3* and *Figure 3.* Seven laboratories (7/34, 21%) received a critical genotyping error:
  - Four laboratories received critical genotyping errors for not reporting one or more of the fusions despite the variant being within the stated scope of the laboratories' assay.
  - Three further laboratories received critical genotyping errors for incorrect reporting of the fusions:
    - One laboratory reported the *FGFR3* fusion as *TACC3::FGFR3.* This is the incorrect orientation and not the clinically active fusion present in the sample.
    - One laboratory reported a *TPM3::ROS1* fusion.
    - One laboratory reported an *FGFR2::TACC13* fusion.
- Several laboratories reported a *MET* exon 14 skipping in this case. This case did not have a *MET* exon 14 skipping variant at the genomic DNA level so would not be picked up by the majority of methodologies however it did have a variant which was present at the cDNA level due to the nature of the artificial material. Testing of *MET* was not requested for this sample and was not assessed.
- Eighteen laboratories (18/34, 53%) received full marks for this case.

Table 3. Laboratory results for each fusion in Case 2.

Fusion	Number of laboratories		
	Detected fusion	Did not report fusion	
EML4::ALK **(exon 13 of *EML4* and exon 20 of *ALK*)**	30 (88%)	Critical error	3 (9%)
		Out of scope	1 (3%)
CD74::ROS1 **(exon 6 of *CD74* and exon 34 of *ROS1*)**	29 (85%)	Critical error	4 (12%)
		Out of scope	1 (3%)
TPM3::NTRK1 **(exon 7 of *TPM3* and exon 10 of *NTRK1*)**	28 (82%)	Critical error	3 (9%)
		Out of scope	3 (9%)
FGFR3::TACC3 **(exon 17 of *FGFR3* and exon 11 of *TACC3*)**	27 (79%)	Critical error	3 (9%)
		Out of scope	4 (12%)

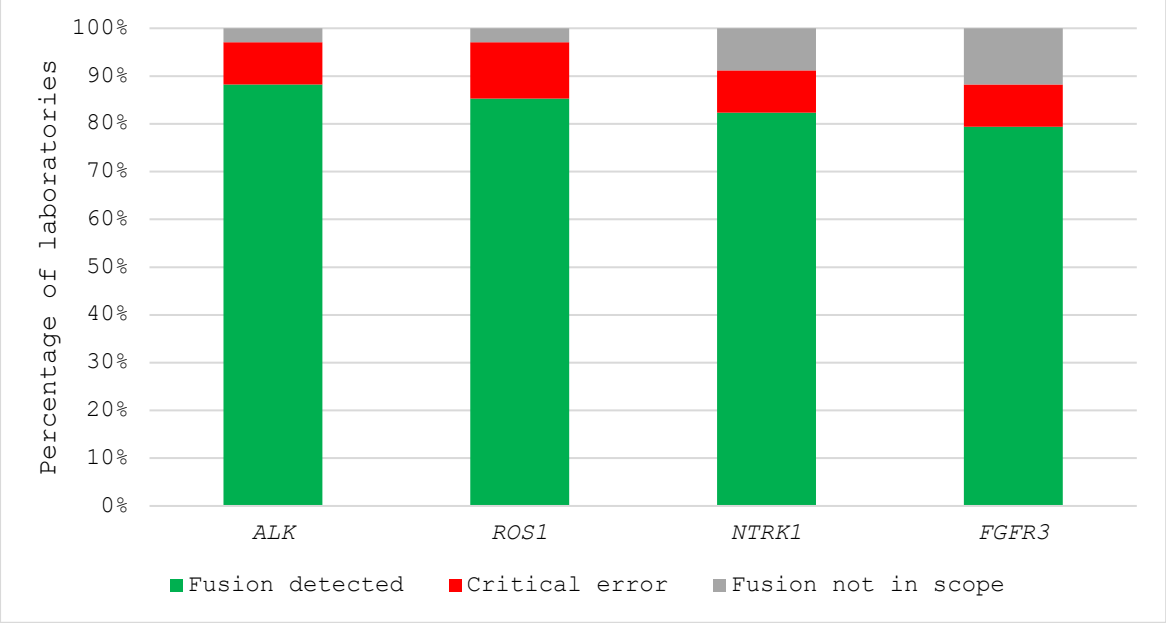

Figure 3. Summary of fusion results in Case 2.

Case 3

Genotyping

- In this case, the genotyping was of a generally high standard, with a mean score of 1.71. The laboratory results for this case are summarised in *Table 4.*
- Two participants did not participate in this case and one participant failed the sample.
- Four laboratories received critical genotyping errors:
  - Three laboratories (3/47, 6%) did not report the *EGFR* variant despite it being within the scope of the laboratories' assay and above the reported LOD.
  - One laboratory (1/47, 2%) reported a *PIK3CA* c.1636C>A; p.(Gln545Lys) at 0.4% VAF. This variant was not detected by validating laboratories or any other participating laboratories.
- Nine laboratories (9/47, 19%) reported the variant as p.(Ser768_Val769insValAlaSer) instead of p.(Ala767_Val769dup). This is incorrect HGVS nomenclature.
- Thirty-three laboratories (70%) received full marks.

Interpretation

- In this case with an *EGFR* insertion in exon 20, it was expected for laboratories to report that this variant has been associated with response to *EGFR* exon 20 specific inhibitor therapy.
- Four laboratories (4/47, 9%) received a deduction for a misleading interpretive comment and one laboratory (1/47, 2%) received a deduction for making no reference to the associated response to *EGFR* exon 20 specific inhibitor therapy.

Case 4

Genotyping

- In this case, the genotyping was of a high standard, with a mean score of 1.93. The laboratory results for this case are summarised in *Table 4.*
- Two participants did not participate in this case.
- One laboratory (1/48, 2%) received a critical genotyping error for not reporting the *BRAF* variant despite it being within the scope of the laboratories' assay and above the reported LOD.
- Forty-four laboratories (92%) received full marks.

Interpretation

- In this case with a *BRAF* variant in a patient with metastatic melanoma, it was expected for laboratories to state that this has been associated with response to BRAF targeted therapy.
- One laboratory (1/47, 2%) received a deduction for a misleading interpretive comment. There were also deductions for providing no interpretation (2/47, 4%), for not indicating which class of inhibitors were recommended (1/47, 2%) and for an incorrect interpretation of the result (1/47, 2%).

Case 5

Genotyping

- This case was generally handled well by laboratories with a mean genotyping score of 1.87. The laboratory results for this case are summarised in *Table 4.*
- Two participants did not participate in this case and one participant failed the sample.
- Two laboratories (2/47, 4%) received critical genotyping errors. One laboratory did not report the *EGFR* c.2369C>T p.(Thr790Met) variant in the sample and one laboratory reported an *EGFR* "c.2386G>A; p.G796S" instead of the p.(Thr790Met) variant.
- Forty-one laboratories (87%) received full marks.

Interpretation

- In this case with both a sensitising variant (deletion in exon 19) and a variant associated with acquired resistance (p.(Thr790Met)) in *EGFR*, it was expected for laboratories provide a statement regarding response to EGFR tyrosine kinase inhibitors (TKIs). Laboratories should have acknowledged that the patient has already received first-line treatment with first generation EGFR TKIs, and the presence of the p.(Thr790Met) variant indicates the patient may benefit from third generation EGFR TKIs[4].
- One laboratory (1/47, 2%) received a deduction for making no reference to the original variant (deletion in exon 19) not being identified in the plasma sample, indicating a likelihood that there may be insufficient tumour DNA in the sample leading to a false negative result. This laboratory was not penalised for their genotype as the variant was below their LOD.

Table 4.Laboratory results for variants in Case 3, 4 and 5.

Case	Variant	VAF Range (Average)	Number of laboratories		
			Detected variant	Did not report variant	
3	***EGFR* c.2303_2304insTGTGGCCAG p.(Ala767_Val769dup)**	**Expected 4%** 2.3-7.5% (4.1%)	42 (89%)	Critical error	3 (6%)
				Below LOD	1 (2%)
				Out of scope	1 (2%)

4	***BRAF*** **c.1799T>A p.(Val600Glu)**	**Expected 1%** 0.3-1.5% (0.8%)	45 (94%)	Critical error	1 (2%)
				Below LOD	1 (2%)
				Out of scope	1 (2%)
5	***EGFR*** **c.2238_2252del p.(Glu746_Thr751delinsAla) (deletion in exon 19)**	**Expected 1%** 0.3-3.2% (0.9%)	45 (96%)	Below LOD	2 (4%)
	EGFR **c.2369C>T p.(Thr790Met)**	**Expected 0.5%** 0.3-1.0% (0.6%)	43 (91%)	Critical error	2 (4%)
				Below LOD	2 (4%)

Organisation

Various aspects of this EQA may be subcontracted, including material preparation by commercial reference material providers and biobanks, assessment by qualified experts and sample distribution. When subcontracting occurs, it is placed with a competent subcontractor and IQN Path is responsible for the work.

Six EQA providers collaborated to supply this pilot EQA on behalf of IQN Path:

EQA provider	Contact information
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Final comments

- The IQNPath cfDNA multibiomarker EQA Project Group would like to thank all participants for their hard work, prompt return of results and their co-operation during this exercise. We would also like to thank our commercial partners in the pharmaceutical industry for their support.
- The purpose of the EQA service is to educate and facilitate the raising of standards.
- We look forward to your participation in the 2025 EQA; please look out for further communications from your EQA provider regarding the opportunity to register for the next EQA round.

Authorisation

This document has been authorised by:

Tracy Stockley, FCCMG, FACMG

Canadian Biomarker Quality Assurance (CBQA)

References

1. HGVS Sequence Variant Nomenclature. http://varnomen.hgvs.org/
2. Bruford EA, Antonescu CR, Carroll AJ, *et al*. HUGO Gene Nomenclature Committee (HGNC) recommendations for the designation of gene fusions. Leukemia. 2021;35(11):3040-3043. doi:10.1038/s41375-021-01436-6
3. ISO 15189:2022. Medical laboratories – requirements for quality and competence.
4. Hendriks LE, Kerr KM, Menis J, *et al*. Oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up☆. Annals of Oncology. 2023;34(4):339-357. doi:10.1016/j.annonc.2022.12.009

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Appendices

1. Participation

Sixty laboratories from 17 different countries (*Figure 4* and *Table 5)* were selected to participate in this pilot EQA for multiple biomarker testing in cfDNA:

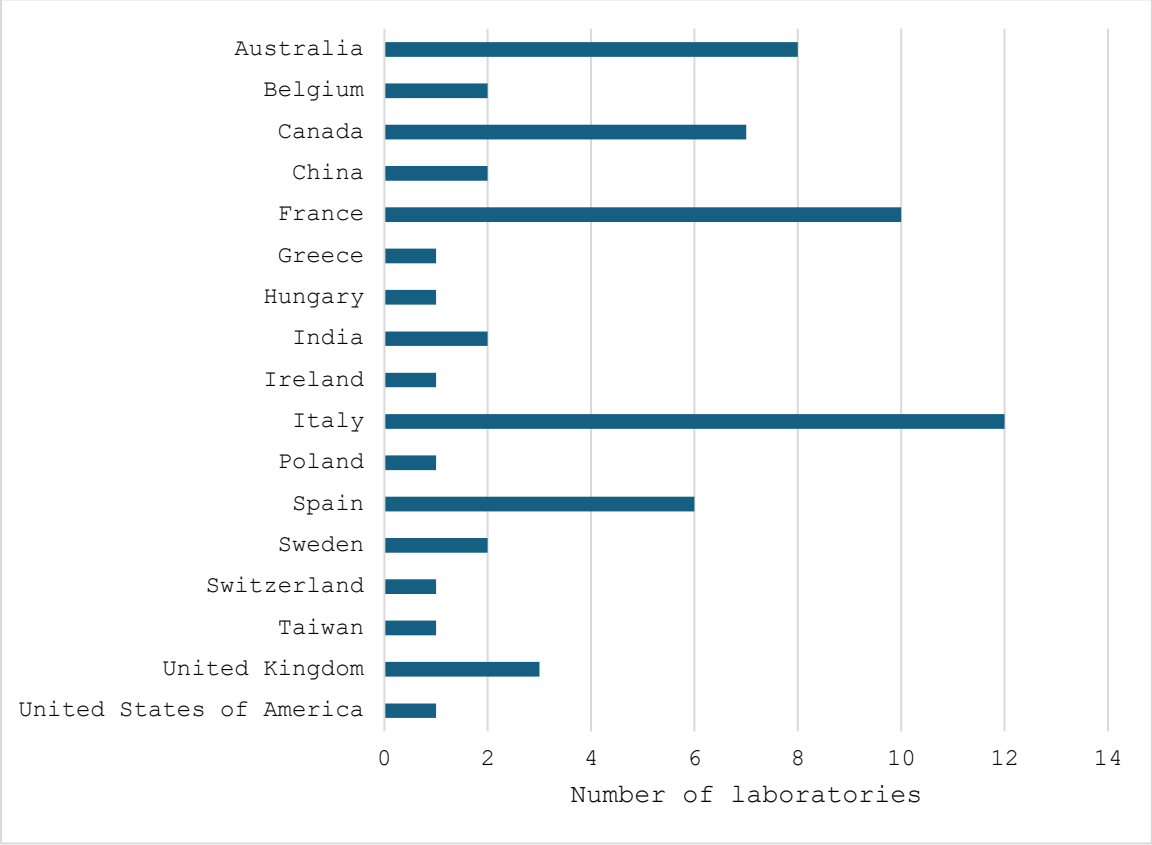

Figure 4. EQA participation by country.

Table 5. EQA participation by EQA provider.

	Number of Registrants	Withdrawn	Did Not Submit	Final Number of Participants
AIOM	10	1	1	**8**
CBQA-PCAB	7	1	1	**5**
EMQN	12	0	0	**12**
GenQA	13	1	0	**12**
Gen&Tiss	10	1	1	**8**
RCPAQAP	8	3	0	**5**
Totals	**60**	**7**	**3**	**50**

2. Sample Validation

Samples used in this pilot EQA were analysed by three laboratories accredited for variant testing in cfDNA, using three different validated testing methodologies (*Table 6*) prior to the EQA distribution. This analysis was conducted to verify the genotype of each case and to ensure suitability of the samples for testing across a range of testing platforms and analytical pipelines.

Table 6. Methods used for validation testing.

Validation Laboratory	Testing Kit
1	Illumina TruSight Oncology 500 ctDNA assay
2	Oncomine Precision Assay (OPA) Gx
3	Guardant360

3. Evaluation Criteria

During this assessment, marking deductions were applied consistently by all EQA providers using a pre-defined set of criteria (*Table 7*). The full score available for each category was 2.00. The interpretation of results the laboratory provided in their reports, and clerical accuracy were only assessed for cases 3, 4 and 5.

Table 7. EQA Marking Criteria

Case	Category	Criterion	Deduction
All Cases	Genotyping	Correct result reported	0.0
		Correct result within limitations of the test performed	0.0
		Critical genotyping error	2.0
		Genotype mis-positioned or mis-called (e.g. incorrect base/amino acid detected)	1.0
		Variant only reported at the protein level. As this is a DNA based test it should also be reported at the nucleic acid level	0.5
		HGVS nomenclature not used / incorrect HGVS nomenclature	0.5
		Minor HGVS error	0.0
		Reference sequence is missing / incorrect / inconsistent	0.1 / 0.5[a]
		Test failure giving no result for the sample and did not state that a repeat sample should be requested	0.5
		Test failure giving no result for the sample and stated that a repeat sample should be requested	0.0
		Not marked	0.0
		Withdrawn from scheme	0.0
3, 4, 5	Interpretation	All essential interpretative elements provided	0.0
		Critical interpretation error	2.0
		Misleading interpretive comment	1.0
		No patient specific clinical interpretation given / same generic interpretation used for every case irrespective of the genotype	0.5
		The report should recommend that a tissue biopsy or repeat sample should be sent for testing if possible[b]	0.5
		The report should state that it is possible that the levels of circulating tumour DNA in this sample may be too low to detect a potential variant[b]	0.5
		Over / inappropriate interpretation of a negative (or normal) results using cfDNA[b]	0.5
		No statement about the methodology performed	0.5
		Failure to provide any, or insufficient, details of the scope of the test and/or limitations of the test performed, in relation to the suitability of the material provided	0.5
		Insufficient information provided on the NGS testing methodology - platform, and/or manufacturer, and/or strategy (ie, WES, targeted), depth, coverage	0.5
		Not marked	0.0
		Withdrawn from scheme	0.0
	Clerical Accuracy	All essential patient identifiers present and no significant clerical errors	0.0
		The patient identifier(s) are insufficient for this case (minor error where patient identity is not in doubt)	0.5
		The patient identifier(s) are incorrect for this case (major error where patient identity is in doubt e.g. date of birth incorrect, patient name incorrect / spelling mistake)	0.5
		Failure to provide sample identifiers (REF and/or LOT number)	0.5

		The sample type should be given on the report	0.5
		The sample type is incorrect	0.5
		Both the date of sampling/receipt and the reporting date should be given on the report	0.5
		The full reason for referral should be included in the report	0.5
		Incorrect pagination	0.5
		Failure to provide correct pagination e.g. pagination missing or only states Page 1 instead of Page 1 of 1 etc	0.0
		There is no evidence that the report was authorised / signed	0.0
		The report should be anonymised.	0.0
		The essential clinically relevant information is lost in this long report. Consideration should be given to reducing the length of your reports.	0.0
		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	0.0
		Clear and concise report	0.0
		Not marked	0.0
		Withdrawn from scheme	0.0
Case	**Category**	**Criterion**	**Deduction**
3	**Interpretation**	The report should state that the EGFR variant in this case has been associated with response to EGFR exon 20 specific inhibitor therapy	0.5
4	**Interpretation**	The report should state that the BRAF variant in this case has been associated with response to BRAF targeted therapy	0.5
5	**Interpretation**	No statement regarding response to EGFR tyrosine kinase inhibitors	0.5
		Interpretation made in the wrong clinical context. This patient has already received first-line treatment with EGFR TKIs	0.5
		The report should state that the original variant (deletion in exon 19 of EGFR) was not identified in the plasma sample and therefore there is a likelihood that there may be insufficient tumour DNA in the sample leading to a false negative result.[b]	0.5

[a]EQA provider specific deductions, [b]Criteria only applied if no variant reported due to scope or LOD.

4. Summary of Results Statistics

The mean scores for genotyping, interpretation and clerical accuracy for all participating laboratories are given below in *Table 8*.

Non-participating laboratories were not marked nor included in this data.

Table 8. Mean scores of all participating laboratories (maximum score = 2.0).

Category	Case 1	Case 2	Case 3	Case 4	Case 5
Mean Genotyping Score	1.21	1.48	1.71	1.93	1.87
Mean Interpretation Score	NA	NA	1.81	1.81	1.88
Mean Clerical Accuracy Score	NA	NA	1.84	1.89	1.95

5. Methodologies

Primary methods used for variant testing in cfDNA by participating laboratories are summarised in *Table 9*.

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Table 9. Summary of primary methodologies used by laboratories for cfDNA testing.

Approach Method	**Count**
Other	
BioRad ddPCR	5
Custom nCounter XT-Elements panel	1
EasyPGX ready NTRK Fusion	1
Paragon Genomics CleanPlex Oncozoom Cancer Hotspot Panel	1
NGS	
AmoyDX Comprehensive Panel	2
AmoyDx® HANDLE Classic NGS Panel	1
Archer FusionPlex Lung panel	2
AVENIO ctDNA Expanded Kit v2 (Roche)	3
Custom Agilent panel	1
Custom Ampliseq panel	1
Custom Illumina NGS panel	1
Custom NGS panel	5
Custom QIAGEN panel	1
Custom Sophia Genetics panel	2
Custom TWIST panel	1
Diatech Pharmacogenetics Myriapod® NGS Cancer Panel DNA CE-IVD kit	3
Guardant360	2
Hedera Profiling 2 ctDNA test panel	1
Illumina Ampliseq Focus Panel	1
Integrated DNA Technologies Liquid OncoPANEL (xGen 56G Oncology Amplicon Panel v2)	1
Ion Torrent RNA Focus Assay	1
Oncomine Focus Assay	1
Oncomine Lung Cell-Free Total Nucleic Acid Research Assay	3
Oncomine Pan-Cancer Cell-Free Assay	4
Oncomine Precision Assay	10
Oncomine-BRCA Assay CHEF Ready panel	1
TruSight Oncology 500	2
UltraSEEK Lung Panel	1