IQN Path ASBL
61 rue de Rollingergrund
L- 2440 Luxembourg

Pilot EQA for Pan Fusion Gene Testing

Contents

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1st July 2025

Dear Colleague,

Thank you for participating in this pilot external quality assessment (EQA) to assess pan fusion gene testing. This EQA has been provided as an IQN Path collaboration between several External Quality Assessment (EQA) providers including: EMQN CIC, GenQA, QuIP, and Gen&Tiss. The EQA assessment includes the scoring of genotype, interpretation, and clerical accuracy. This EQA summary report includes combined assessment data using harmonised marking criteria. The collection of results, data analysis and preparation of this report was undertaken by EMQN CIC, GenQA, QuIP and Gen&Tiss. The harmonized review of the results has now been completed. This EQA Summary Report is an overarching summary which collates the results from all EQA providers.

Background

Accurate detection and characterisation of gene fusions in solid tumours is of increasing importance due to recent developments in tumour-agnostic therapies. RNA and DNA-based next generation sequencing (NGS), as well as other RNA-based testing strategies, are increasingly being adopted as a means of proficient detection of gene fusions. The development of EQA in parallel with these new developments is essential to assure accurate test results and minimize the risk of real patient harm since evidence from EQA schemes shows that the introduction of any new test is usually accompanied by a high diagnostic error rate (often up to 25%).[1–5] The objective of this pilot study was to establish the feasibility of EQA for pan fusion gene testing.

EQA Design & Purpose

This EQA scheme was designed to test the entire routine diagnostic workflow of a laboratory, from nucleic acid extraction and sample processing to data analysis and variant reporting. Three mock clinical referrals and corresponding formalin-fixed paraffin embedded (FFPE) tissue samples were supplied to participants for testing via their routine diagnostic pipeline.

The aim was to assess the ability of participating laboratories to undertake fusion gene testing in FFPE tissue, for a range of clinically significant fusion variants, involving the following genes: *ALK*, *ROS1*, *RET*, *NTRK1*, *NTRK2*, *NTRK3*, *FGFR1*, *FGFR2*, or *FGFR3*. This included an assessment of testing accuracy and an evaluation of the standard of clinical reporting against three categories: genotyping, interpretation, and clerical accuracy, with the objective of helping laboratories to standardise and improve their reporting. Each category was assessed using a set of pre-defined comments (Appendix 4, Table 3), as agreed by the working group. 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 sections from 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 information and 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.

Participation

One-hundred and twenty laboratories from 30 countries (Appendix 1, Figure 1) registered with EMQN CIC (70); GenQA (30); QuIP (17); and Gen&Tiss (3), were selected to participate in this pilot EQA scheme based on responses in an expression of interest survey. Of the 120 laboratories selected to participate in this pilot EQA scheme, 104 returned results by the assessment deadline, equating to a participation rate of 86.7%.

Samples Provided & Testing Required

Scheme participants were provided with formalin-fixed paraffin embedded (FFPE) samples for pan fusion gene testing via their routine analytical pipeline(s). FFPE embedded cell lines, purchased from a commercial manufacturer (GeneWell, China), were used as reference materials for this EQA. Identical samples were distributed for testing to all participating laboratories regardless of which EQA provider they registered with. All laboratories were supplied with samples from the same batch. Each sample was supplied with a corresponding mock clinical scenario including patient name, date of birth, clinical presentation and test request (Appendix 2, Table 2). Participants were instructed to report results using an online form hosted on the Formdesk app (https://en.formdesk.com) and via direct submission of clinical reports to their respective EQA provider.

Scheme Report on Behalf of the IQN Path Pan Fusion Gene Working Group

All Cases

Genotyping

- The overall standard of genotyping was high across all three cases, with an average score of 1.83 (out of 2.00 possible marks). This is a slight decline on the overall average score of 1.86 achieved in the previous pilot round.
- Overall, three laboratories made five critical genotyping errors. Of the 312 reports that were assessed for genotyping, five (5/312, 1.60%) included a critical genotyping error (Appendix 5, Table 7). Please see feedback on individual cases for further information.
- Participants were instructed to report fusion genes in accordance with nomenclature guidelines from a reputable source such as Human Genome Variation Society (HGVS)[6], the Hugo Gene Nomenclature Committee (HGNC)[7] or the Variant Interpretation for Cancer Consortium (VICC)[8]. Whilst many laboratories chose to report results using the most simplistic form of nomenclature (i.e. following guidelines from HGNC, which does not include exon-level detail or allow for referencing gene transcript accessions), it was apparent from our assessment that quite a large proportion of laboratories reported nomenclature that did not conform with the guidelines of any of these organisations.
- Reports submitted by 15 laboratories (15/103, 14.5%) did not include exon-level detail or breakpoint genomic coordinates of the detected fusion event, even though the chemistry used for testing would allow for reporting this level of information.
- Thirty-four laboratories (34/312, 10.8%) did not include reference transcript accession and/version numbers in their reported fusion gene nomenclature. We recommend the use of MANE given its stability.[9] However, we recognize transcript reporting may be limited by the assay used for testing. Nevertheless, the fusion nomenclature must be correct according to which transcript the genotype is reported against.

- Therefore, we would like to reiterate our advice from the 2023 Pan Fusion Gene Pilot EQA round to a wider cohort of participants: Use of a **standardised** form of nomenclature is **crucial** to avoid ambiguity and facilitate data sharing. The project team recognise that there is wide variation in the complexity of nomenclature recommended for reporting fusion genes. Although more detailed forms (such as those proposed by HGVS) offer a higher level of detail on the fusion detected (and therefore less ambiguity), we recognise that these complicated forms of nomenclature may be difficult for a non-expert in genetics to understand. Whilst HGNC recommendations for describing fusion genes is clear and concise, we strongly recommend providing exon level detail where testing chemistry allows the provision of such information, as this enables a full biological interpretation of the result and accurate determination of clinical actionability. This is especially important following the detection of novel fusions where it is important to understand the protein domains implicated in the fusion and to corroborate potential oncogenicity based on the known function of those domains.
- The project group recommend the use of fusion nomenclature following VICC guidelines since the descriptions are concise, informative and readable; it includes exon level information and transcript accessions. Please see the VICC Gene Fusion Specification Guidelines for further information.
- During our assessment, we noted that ten laboratories (10/312, 3.2%) reported fusion breakpoints using genomic coordinates but did not reference the version of the human genome that the coordinates corresponded to. The genome version is crucial to understand the location of the reported coordinates so should always be included even if alternative nomenclature detailing transcript accessions with version numbers is present in the report.
- Ideally, laboratories would report on whether the detected fusion is in-frame or out-of-frame to allow full interpretation of the potential pathogenicity of the aberration, but we appreciate that this is not always feasible and depends on the kit/chemistry used for testing.
- Please note, according to HGVS guidelines[6], use of a hyphen ('-') between gene names denotes a read-through variant, whereas a double colon ('::') denotes a gene fusion event (https://hgvs-nomenclature.org/stable/recommendations/general/).

Interpretation

- Of the 300 reports assessed for this category, 276 (92.0%) provided an interpretation of the genotype result, which is excellent.
- The average interpretation score across all three cases was 1.91 (out of 2.00), which is very good.
- 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. However, if the detected fusion is associated with an approved targeted therapy, a statement reflecting this information would be acceptable as an interpretive comment.
- Across all three cases, there were five critical interpretation errors awarded to two different laboratories; three were made by the same laboratory in each case for failing to report the result in clinical context and mention the opportunity for targeted therapy. This is an important aspect of clinical interpretation intended to avoid missed treatment opportunities for patients with oncogenic fusion genes. In this pilot scheme round, only two laboratories were assessed as having failed to mention therapeutic options.

- Forty-two reports (42/300, 14.0%) included mention of specific drug names in the interpretation of the fusion gene result. It is preferable to report the class of drugs a patient is eligible for (e.g.- tyrosine kinase inhibitors) to capture all approved drug therapies with the same mechanism of action, and to avoid naming specific brands of drugs which may inadvertently result in excluding patients from newer, equally effective or better tolerated agents. This also allows clinicians to choose the most appropriate treatment option for their patient (i.e.- taking in to account central nervous system penetration if relevant, toxicity profiles, or the presence of resistance mutations).[10] It is equally important to note that drug availability varies by country and the market evolves quickly.
- Of the 300 reports assessed for interpretation, 84 were evaluated as having no or insufficient information about test limitations. Information such as scope of testing (e.g. genes included), analytical sensitivity and specificity, 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. This year, because the samples used in each case contained an oncogenic fusion, no marking deduction was applied for this error.
- Across all three cases, 23 reports (23/300, 7.66%) did not contain information about the assay/testing method used and/or the scope of testing performed. This information is essential for understanding test accuracy, interpretability and informing clinical decision-making, and should always be included on a clinical report.
- It was apparent during the assessment of reports submitted to this EQA round that reporting on quality metrics from fusion gene testing varies hugely and that laboratories require some guidance in this area to improve standardisation, clinical validity and safety of reporting. Ideally, reports should include information on:
    - Technical characteristics, for example: analytical sensitivity (limit of detection; LOD); analytical specificity (i.e.- the test's ability to detect only the intended targets, avoiding false positives from background "noise" or non-specific amplification); reproducibility and precision; and accuracy.
    - Clinical performance, for example: clinical sensitivity and specificity (i.e. the proportion of true positive or true negative results correctly identified); positive/negative predictive value (i.e. likelihood that the positive/negative result is true); and details about the population used for validation (i.e. cohort description, cancer types, fusion events, sample numbers).
    - Test quality metrics, for example: read depth/coverage, on-target rate, mapping quality metrics, internal/quality controls.
    - Test/reporting limitations, for example: regions/fusions not covered, assay limitations, and potential sources of false positives/negatives (e.g.- pseudogenes, low/high complexity regions).
- The project team anticipates, through continued provision of this scheme, it will be possible for EQA providers together with their teams of expert assessors, to provide further guidance on minimum requirements of reporting on quality of fusion gene testing, particularly RNA-based NGS methods.

Patient Identifiers and Clerical Accuracy

- The average score for clerical accuracy was 1.88 (out of 2.00) across all three cases, which is good. However, there were some recurrent errors:
    - Clear and accurate identification of the patient undergoing testing is a crucial element of the reporting process.[11] It is recommended that this information is included on each page of a multi-page report in case the pages become separated.[11]
    - Twenty-seven reports (27/303, 8.9%) did not specify the patients' sex. This is important for quality control (e.g.- identification of sample swaps), and establishing gender-

specific pathogenicity (for example, related gender specific risks, as well as differences in fusion gene expression across genders due to tissue specificity[12]).
- Forty-four reports (44/303, 14.5%) 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.
- Fifty-seven (57/303, 18.8%) had incorrect or missing pagination. All pages of a report should include correct pagination, in a format which includes the total number of pages (i.e.: 1 of 2; 2 of 2) such that the reader understands how many pages make up the report in its' entirety, and whether any pages are missing.
- Forty-one (41/303, 13.5%) reports did not include a signature indicating the report has been authorised. Every report should include two signatories: one from the individual who interpreted the data and prepared the report, and a second from an appropriately qualified individual who served to check the information, thereby authorising its content and conclusions.[11,13]
- Thirty-three (33/303, 10.9%) did not include dates of sample receipt/testing/reporting, or the type of sample tested (i.e.- solid tumour FFPE). Reports should also detail the block ID and section number of the specimen tested as a mean of providing a unique identifier for the sample of which testing was performed.

Case 1

Genotyping

- The sample provided with this case had a *FGFR2*::*COL14A1* fusion.
- The average score was 1.86, which is very good. Of the 104 laboratories that participated in this case, 59 (56.7%) received full marks for genotyping.
- Fifteen laboratories (15/104, 14.4%) failed to include or used incorrect/inconsistent gene transcript accessions in their reports.
- Five laboratories (5/104, 4.8%) did not provide exon-level detail or genomic coordinates of the reported fusion, even though the technology they used for testing would have allowed.
- Two laboratories received major nomenclature errors (-0.50 marks): one for failing to list the 5' fusion partner first in the nomenclature description, and another for mis-reporting the exons involved in the fusion event.
- There were two critical genotyping errors awarded to laboratories that failed to detect the *FGFR2*::*COL14A1* fusion, despite stating it was within the scope of their testing strategy.
- Seventeen laboratories (17/104, 16.3%) failed to detect the clinically actionable fusion present in this sample due to limitations of their testing strategy. This statistic highlights the limited diagnostic proficiency of some amplicon-based off-the-shelf fusion gene testing kits. Reports should clearly state the scope of testing performed, including exactly which fusion events/partners can be detected based on the chemistry used for testing. Where testing has failed to identify an oncogenic fusion, a statement along the lines of "*Negative results do not entirely rule out the presence of an oncogenic fusion beyond the scope of the current strategy*", should be included.

Interpretation

- The average score for interpretation was 1.86; of the 99 laboratories assessed for interpretation in this case, 88 (88.8%) received full marks, which is excellent.
- Four laboratories (4/99, 4.04%) failed to provide a clinical interpretation of the result and consequently received a deduction of 1.50 marks.

Case 2

Genotyping

- The sample provided with this case had an *EML4*::*ALK* fusion.
- The average score in this case was 1.83, which is very good. Of the 104 laboratories that participated in this case, 49 (47.1%) received full marks for genotyping.
- Twenty-seven laboratories (27/104, 30.1%) failed to include the gene transcript accession alongside the fusion nomenclature or used an incorrect/inconsistent transcript accession.
- Four laboratories (4/104, 3.85%) received major nomenclature errors (-0.50 marks) for reporting a mis-positioned genotype at the exon level.
- Eight laboratories (8/104, 7.7%) received minor nomenclature errors (-0.2 marks) for reporting a mis-positioned genotype at the cDNA coordinate level.
- There were two critical genotyping errors: one false negative, and one false positive result (see Appendix 5, Tables 5 and 7).

Interpretation

- The average score for interpretation in this case was 1.93, which is excellent; 94 out of 100 (94.0%) laboratories assessed for interpretation received full marks.
- Three laboratories (3/100, 3%) did not provide a clinical interpretation of the result.
- One laboratory received a critical interpretation error for failing to mention eligibility for ALK-targeted therapies.

Case 3

Genotyping

- The sample provided with this case had a *SLC34A2*::*ROS1* fusion.
- The average score for genotyping was 1.80; of the 104 laboratories that participated, 45 (42.3%) received full marks in this category.
- Thirty laboratories (30/104, 28.9%) failed to include the gene transcript accession alongside the fusion nomenclature or used an incorrect/inconsistent transcript accession.
- Twelve laboratories (12/104, 11.5%) received a major nomenclature error, whilst a further 19 laboratories (19/104, 18.3%) received a minor nomenclature error.
- One laboratory received a critical genotyping error due to reporting a false positive result (see Appendix 5, Table 7 for details).
- Twenty laboratories (20/104, 19.2%) reported the presence of multiple *SLC34A2*::*ROS1* fusion isoforms; up to four in some cases.

Interpretation

- The average score for interpretation was 1.93; 96 out of 101 laboratories (95.5%) assessed for interpretation received full marks.
- Three laboratories (3/101, 2.97%) did not provide a clinical interpretation of the result.
- One laboratory (1/101, 0.99%) received a critical interpretation error for failing to report the result in the context of eligibility for ROS1-targeted therapies.
- Whilst the presence of multiple isoforms is biologically plausible due to alternative splicing or the generation of multiple breakpoints during a chromosomal rearrangement (that leads to the formation of a fusion gene), few if any of the laboratories that reported this offered an explanation as to the biological or clinical relevance of multiple isoforms, which could leave the reader confused or open to making their own judgement. This is important because not all alternative isoforms may

be oncogenic, it depends which domains the product contains, or they may confer different levels of oncogenic activity. Whilst the presence of alternative isoforms can explain differences in drug sensitivity, drug resistance or prognosis, evidence of the impact of specific isoforms in the presence of others is generally lacking.[14–16] Multiple isoforms may also arise as an artifact of testing or bioinformatics analysis. Therefore, they should be reported in the context of available evidence and preferably only after confirmation by an alternative method.

Professional standards

Laboratories are assessed against the guidelines, relevant peer reviewed literature and currently available references. Other guidelines against which laboratory reports are assessed may include the international nomenclature HGVS[6], HGNC[7], and VICC[8] as well as ISO standards (ISO15189).[13]

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.

Three EQA providers collaborated to supply this pilot scheme on behal of IQN Path:

EQA provider	Contact information
emqn	EMQN CIC, 3rd Floor ICE Building, 3 Exchange Quay, Salford, M5 SED, United Kingdom. **Tel:** +44 161 757 1591 6741 **Email:** office@emqn.org
GenQA Genomics Quality Assessment Member of UK NEQAS consortium	GenQA, Laboratory Medicine, NHS Lothian NINE, Edinburgh BioQuarter Little France Road, Edinburgh, EH16 4UX, United Kingdom. **Tel:** +44 131 242 6898 **Email:** info@genqa.org
QuIP Quality in Pathology	Qualitätssicherungs-Initiative Pathologie QuIP GmbH, Reinhardtstraße 1, 10117 Berlin, Germany. **Tel:** +49 30 921 0717 0 **Email:** office@quip.eu
gen&tiss	Hautepierre Hospital 67098 Strasbourg cedex **Tel:** +33 (0)3 88 12 81 41 **Email:** secretariat@genetiss.org

IQN Path ASBL
61 rue de Rollingergrund
L- 2440 Luxembourg

Final comments

- The IQN Path Fusion Gene 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 future 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:

Dr. Simon Patton on 1st July 2025

CEO

Amendments to this summary EQA report

Version	*Page*	*Section*	*Change*	*Published*
1	-	-	*None*	*1st July 2025*
2				
3				

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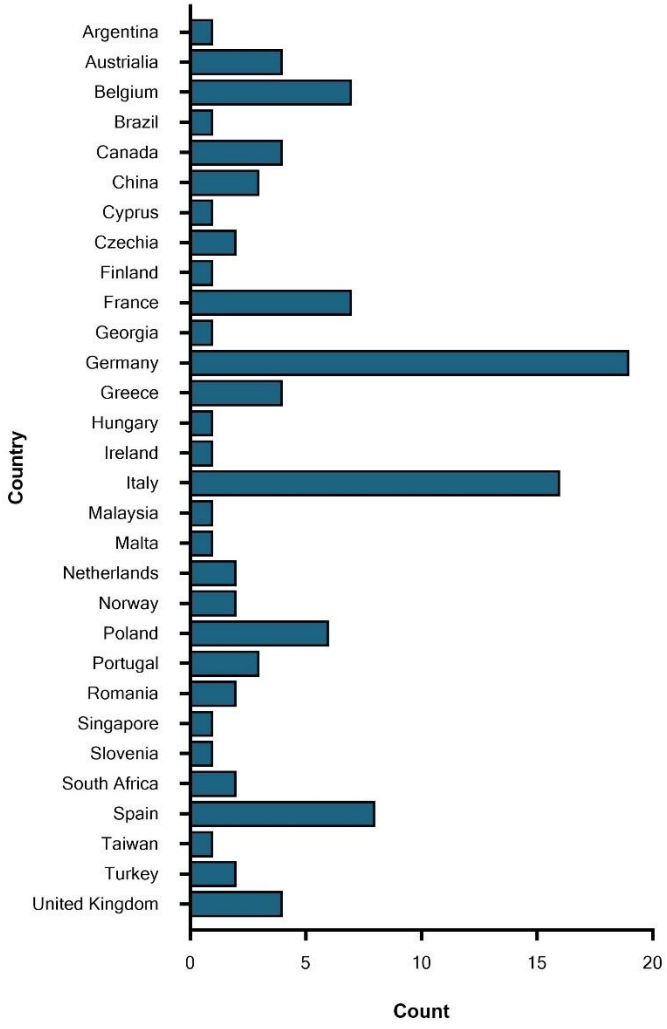
IQN Path ASBL
61 rue de Rollingergrund
L- 2440 Luxembourg

Appendices

1. Participation

120 laboratories from 30 different countries were selected to participate in this pilot EQA scheme for pan fusion gene testing:

Figure 1: Participating Countries



	No. Registrants	Withdrawn/DNS	Final No. Participants
EMQN	70	6	**64**
GenQA	30	5	**25**
QuIP	17	5	**12**
Gen&Tiss	3	0	**3**
Totals	**120**	**16**	**104**

Table 1: Participant Numbers by EQA Provider for the Pan Fusion Gene Pilot EQA
No.: number; DNS: did not submit

2. Samples Provided and Validated Results

Case	Name	Sex	Date of Birth	Referral Reasons	Nomenclature Guidelines	Validated Genotype Result*
1	Dali ALFARO	Male	08/01/1970	Dali was admitted to hospital with complaints of severe right upper abdominal pain and lower abdominal distension. Examination found an enlarged cervical lymph node on the left (sized at 2 x 2.3 cm^2) with undefined margins. Computed tomography (CT) scan revealed a large unresectable tumour on the left lobe of the liver and peritoneal dissemination. Liver tumour biopsy confirmed intrahepatic cholangiocarcinoma. No likely causal variants were identified in *IDH1* and the tumour was shown to be MMR proficient/MSI stable. Fusion gene testing has been requested to inform clinical management.	**HGNC**	*FGFR2*::*COL14A1*
					VICC	NM_000141.5(*FGFR2*):e.17::NM_021110.4(*COL14A1*):e.34†
					HGVS	NM_000141.5:r.-633_2301::NM_021110.4:r.4078_*2342††
2	Ebbe DASTRUP	Male	11/10/1982	Ebbe, a 42-year-old infrequent smoker of 10 years, was admitted to hospital with posterior chest pain and intermittent fever. Examination revealed an elevated temperature of 38.5°C, decreased breath sounds in the right upper lung, intermittent cough and wheezing. Contrast-enhanced chest computed tomography (CT) showed a 4 cm^2 irregularly shaped mass in the right upper lobe that was confirmed as active by positron emission tomography (PET). Transbronchial biopsy revealed non-small cell lung carcinoma (NSCLC) by immunohistochemistry (IHC). First-line genetic testing did not reveal any oncogenic alterations in *EGFR, KRAS, BRAF* and *ERBB2*. Further molecular testing for oncogenic fusion genes is requested to inform therapeutic interventions.	**HGNC**	*EML4*::*ALK*
					VICC	NM_019063.5(*EML4*):e.13::NM_004304.5(*ALK*):e.20
					HGVS	NM_019063:r.-259_1489::NM_004304.5:r.3173_*451
3	Sedo DE VOSS	Male	03/05/1966	Sedo is a 58-year-old non-smoker who was referred for investigation upon presentation with chest pain, persistent cough and unexplained weight loss. A suspicious mass was detected in the peripheral lung tissue upon thoracic computed tomography (CT) scan and stage IV lung adenocarcinoma was diagnosed from histological analysis of the resected tumour. Lymph node metastasis in both sides of the neck was observed following surgery despite the patient undergoing two cycles of adjuvant chemotherapy. Molecular profiling was negative for driver mutations in *EGFR, KRAS, BRAF* and *ERBB2*. Further testing for oncogenic driver fusion genes has been requested with a view to the implementation of targeted therapy.	**HGNC**	*SLC34A2*::*ROS1*
					VICC	NM_006424.3(*SLC34A2*):e.4::NM_001378902.1(*ROS1*):e.33**
					HGVS	NM_006424.3:r.-43_379:: NM_001378902.1:r.5231_*1139

Table 2: EQA Sample Details and Validated Results.

**Where exon 1 contains the ATG start codon and subsequent exons are numbered systematically
†*FGFR2* has a designated MANE Plus Clinical transcript, therefore the following VICC nomenclature was also accepted: NM_022970.4(*FGFR2*): e.17::NM_021110.4(*COL14A1*):e.34
††*FGFR2* has a designated MANE Plus Clinical transcript, therefore the following HGVS nomenclature was also accepted: NM_022970.4:r.-647_2304::NM_021110.4:r.4078*2342
**Case 3: Nomenclature involving the alternative ROS1 transcript NM_002944.2 was also considered acceptable:

- VICC: NM_006424.3(*SLC34A2*):e.4::NM_002944.3(*ROS1*):e.32
- HGVS: NM_006424.3:r.-43_379::NM_002944.3:r.5249_*1139

3. Evaluation Criteria

During this assessment, marking deductions were applied consistently by all EQA providers using a pre-defined set of criteria.

Case	Category	Criterion	Deduction
All Cases	Genotyping	Correct result reported	0
		Correct result within limitations of test	0
		Critical genotyping error	2
		Major nomenclature error	0.5
		Minor nomenclature error. Please see comments and/or the scheme report for further information.	0.2
		Exon-level detail /breakpoint genomic coordinates of fusion not provided. Please see comments and/or the scheme report for further information.	0.2
		Genomic descriptions should reference the genome version	0.2
		Reference sequence is missing / incorrect / inconsistent	0.2
		Reference sequence version / transcript number is missing / incorrect / inconsistent	0.2
		Use of MANE transcripts is preferred- see report	0
		HGCN nomenclature used	-
		HGVS nomenclature used	-
		No nomenclature guidelines followed	0.2
		Fusion partner not characterised when technology permits	0.5
		Fusion partner not characterised due to technology limitations	
		Not tested	-
		Test Failed	-
		Not marked	-
		Withdrawn from scheme	-
	Interpretation	Clinical Interpretation provided	0
		Critical interpretation error	2
		No clinical interpretation provided	1.5
		It is not advisable to use specific drug names; drug classes are preferable	0
		Failure to mention therapeutic options	1
		Report should state that no fusions detected that are related to known therapy for clinical presentation	0.5
		No information about test limitations. No deduction made as a pathogenic variant has been identified	0
		No statement about the assay/testing method used and/or scope of testing performed.	0.2
		Not tested	-
		Test Failed	-
		Not marked	-
	Clerical Accuracy	All essential patient identifiers present and no significant clerical errors	0
		No restatement of the reason for patient referral	0
		DOB incorrect or missing	1
		Patient name has a spelling error	0.5
		Patient gender is not specified/is incorrect	0.5
		The title of your report is misleading / absent	0

		Failure to provide patient identifiers on each page of the report	0
		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
		Failure to provide the dates of sample receipt / testing or reporting	0
		Failure to provide the sample type	0.5
		The sample type provided is incorrect	0.5
		Neoplastic cell content incorrect	0
		No block number provided	0.5
		No section ID provided	0
		Failure to indicate an authorising signature	0
		Incorrect/missing pagination	0.5
		Recommended format for pagination is "Page 1 of 1", "Page 1 of 2", etc.	0
		Failure to provide a clear presentation of results	0
		Long report, a one or two page document with the essential information is preferred	0
		Clear and concise report	0
		Not marked	-
		Withdrawn from scheme	-
Case	**Category**	**Criterion**	**Deduction**
3	**Interpretation**	Multiple ROS1 fusion transcripts reported	0.0

Table 3: EQA Marking Criteria

4. Summary of Results Statistics

The mean scores for genotyping/analytical, interpretation, clerical accuracy and the total mean score for all participating laboratories are given below in Table 4. A summary of the number of critical errors per case is provided in Appendix 6, Table 5 -Table 7.

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

Category		Case 1	Case 2	Case 3
Genotyping	Mean (SD)	1.86 (0.31)	1.83 (0.31)	1.80 (0.28)
	Median (SD)	2.0 (0.31)	1.8 (0.31)	1.8 (0.28)
Interpretation	Mean (SD)	1.86 (0.47)	1.93 (0.32)	1.93 (0.32)
	Median (SD)	2.0 (0.47)	2.0 (0.32)	2.0 (0.32)
Clerical Accuracy	Mean (SD)	1.88 (0.29)	1.88 (0.28)	1.88 (0.28)
	Median (SD)	2.0 (0.29)	2.0 (0.28)	2.0 (0.28)

Table 4: Mean and Median Genotyping Scores with Standard Deviation (SD).

	Case 1	*Case 2*	*Case 3*	*Totals*
No. labs participating	104	104	104	312
No. labs with full marks	59	49	45	153
% full marks	56.73	47.12	43.27	49.03
Critical error	2	2	1	5
% error	1.92	1.92	0.96	1.60

Table 5: Critical Genotyping Errors

	Case 1	Case 2	Case 3	Totals
No. labs participating	99	100	101	300
No. labs with full marks	88	94	96	278
% full marks	88.9	94.0	95.0	92.7
Critical error	3	1	1	5
% error	3.03	1.00	0.99	1.67

Table 6: Critical Interpretation Errors

5. Critical Genotyping Error Summary

Case	Count	Error
1	2	False negative result: Failure to report: NM_000141.5(*FGFR2*):e.17::NM_021110.4(*COL14A1*):e.34
2	1	False negative result: Failure to report: NM_019063.5(*EML4*):e.13::NM_004304.5(*ALK*):e.20
	1	False positive result: *SLC34A2::ROS1* fusion reported (likely sample swap)
3	1	False positive result: *EML4::ALK* fusion reported (likely sample swap)

Table 7: Summary of Critical Genotyping Errors (CGE) made in this EQA Scheme

6. Methodologies

Primary methods used for pan fusion gene testing by participating laboratories:

Approach Method	Count
RT-qPCR	
Biocartis GeneFusion Assay IVD	1
Biocartis GeneFusion Assay RUO	4
NGS	
Agilent Sureselect Cancer RNA CGP	1
Archer™/IDT: FusionPlex® Comprehensive Thyroid & Lung (CTL) Kit and NEBNext® Ultra II Directional RNA Library Prep Kit	1
AmoyDx HANDLE Classic NGS Panel	2
AmoyDx Pan Lung Cancer PCR Panel	1
Archer FusionPlex lung v1	2
Archer FusionPlex lung v2	10
Archer FusionPlex Pan Solid Tumor v1	3
Archer FusionPlex Pan Solid Tumor v2	1
Archer FusionPlex Sarcoma v2	1
Diatech Myriapod Cancer Probe Plus (NG101)	1
Diatech Pharmacogenetics Myriapod NGS Cancer panel RNA	3
Illumina Ampliseq for Illumina Focus Panel	2
RNA enrichment prep with the exome capture oligos	1
Illumina Pancancer fusion panel	1
Illumina TruSight Oncology 500	4
Illumina TruSight RNA Fusion Panel	1
Illumina TruSight RNA Pan-Cancer Panel	3
Illumina TruSight Tumor 170	1
Illumina TruSeq RNA Exome	1
Illumina TruSeq Stranded Total RNA	2
Ilumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus	1
AmpliSeq for Illumina RNA Fusion Lung Cancer Research Panel	1
Qiagen QIAseq Human Lung Cancer Multimodal Panel	1
Qiagen RNA Fusion XP Lung Cancer Panel	1
Qiagen RNA Fusion XP Solid Tumor Panel	1
QIAseq Targeted RNAscan Custom Panel	2
QIAseq FastSelect RNA Removal Kit (NEBNext Ultra II Directional RNA Library Prep Kit)	1
Roche AVENIO Tumor Tissue Expanded Kit	1
Roche Kapa HyperPETE LC Fusion Panel	2
Ion AmpliSeq™ RNA Fusion Lung Cancer Panel	2
Life Technologies Oncomine™ Childhood Cancer Research Assay	1
Life Technologies Oncomine™ Comprehensive Assay	6
Life Technologies Oncomine™ Comprehensive Assay Plus	6
Life Technologies Oncomine™ Dx Express Test	2

Life Technologies Oncomine™ Dx Express Test Panel	1
Life Technologies Oncomine™ Focus Assay	10
Life Technologies Oncomine™ Precision Assay	15
Life Technologies Oncomine™ Precision Assay GX	1
Life Technologies Oncomine™ Focus Assay	1
Twist RNA Targeted Sequencing	1
Custom-design	5

Table 8: Approaches to Testing and Methods Used

7. Test Scope and Limitations Parameters which May be Included in a Clinical Report

Current best practice guidelines[17, 13,18,19] recommend that the following be included:

Item	Description
What material has been tested?	e.g., RNA extracted from FFPE was tested
Minimum neoplastic cell content (NCC) required for the assay	e.g., >20%
What tests were performed?	Define the (horizontal) extent of testing e.g., sequence analysis of all exons and flanking sequences (+/- 20bp) of the genes were analysed.
The method used to perform the tests	e.g., NGS, RT-PCR etc.
Limit of Detection (LOD)	Ideally this should be described as the % of mutant allele that is detectable in a wild-type background. This should be experimentally determined during the assay validation process. If derived from a kit pack insert, then this should be verified in your laboratory.
Analytical scope	A brief summary of the test used and what the laboratory is trying to achieve: What does you test cover e.g., does your test detect all types of variants or are some often missed e.g., indels >15bp, variants in regions of homology or next to homopolymer tracts, large exon rearrangements causing copy number changes (deletions/duplications)?
Clinical yield	What proportion of actionable variants the test detects. The testing strategies provided by the laboratory should be evaluated periodically by authorised personnel to ensure they are clinically appropriate for the test requests received. Any results provided that are considered to be preliminary should be identified in the clinical report.
Analytical sensitivity	Defined by the read depth (vertical coverage)
NGS details	The chemistry/platform used along with details of any kits and the regions/genes covered if appropriate.
NGS sequencing depth	Depth of a genomic position is equal to number of reads aligned to that position, however not every base can be listed on the report so a minimum depth may be provided.
NGS horizontal coverage	Horizontal coverage, given by the percentage of the region of interest (target) meeting the laboratory's minimum read depth, e.g., 99% of the target generated sequence at a minimum read depth of 20x. This must be given for the whole target (panel). It is also recommended to make this information available for individual genes, either in the report or in a separate technical report, or to say that the data is available via a web link, or upon request.

Table 9: Test Scope and Limitations Parameters which May be Included in a Clinical Report