Assessing the Quality of cfDNA Somatic Variant Testing in Clinical Practice

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Introduction

Implementation of novel tests require assurance of test accuracy and appropriate reporting to ensure patient safety. As cell free DNA (cfDNA) somatic variant testing is adopted globally then external quality assessment (EQA) is required to demonstrate the quality of the clinical service provided and can deliver tailored expert guidance where required. However, the delivery of such schemes is challenging due to specific sample requirements and low allelic frequencies required to replicate patient samples.

Methods

Objectives

The aim of the EQAs was to assess the **testing accuracy** and **clinical reporting** of *EGFR* and *KRAS** variants in cfDNA in lung cancer and help make **improvements** using a combination of assessment and feedback (expert commentary).

** 2020 EQA did not include assessment of KRAS variants*

Participating laboratories were required to:

- Correctly determine the genotype of the samples provided,
- Interpret the results in response to the clinical referral in a clear format,
- Correctly use internationally accepted standard nomenclature[1],
- Provide appropriate and accurate patient and sample information and identifiers.

Format

The EQA format is displayed as Figure 1.

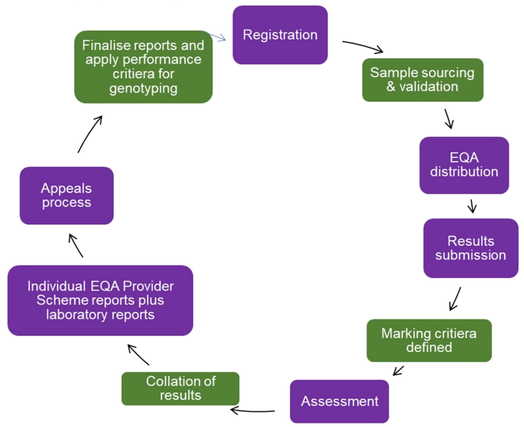

Figure 1 – Summary of the EQA format

EQA cases and samples

Bespoke artificial plasma samples with a range of common *EGFR* and *KRAS** pathogenic variants at defined allelic frequencies were distributed to participants for cfDNA testing and reporting in the context of non-small cell lung cancer.

The participants received three custom manufactured artificial plasma samples with mock clinical referrals for testing (Table 1). Each sample contained 80ng/ml of cfDNA and 3ml of sample was provided. The manufacturer validated the samples using digital droplet PCR (ddPCR) and the genotype was validated independently by the EQA providers in three different laboratories by Roche cobas® EGFR Mutation test v2 assay, ddPCR using custom BioRad primers and an in-house next generation sequencing (NGS) panel (capture based).

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Conflicts of interest: The authors have no conflicts of interest to declare.

References: 1. HGVS Sequence Variant Nomenclature. http://varnomen.hgvs.org

Methods

Participants

Two EQA providers, Genomics Quality Assessment (GenQA) and European Molecular Genetics Quality Network (EMQN) CIC invited molecular pathology testing laboratories to participate in annual rounds of EQA.

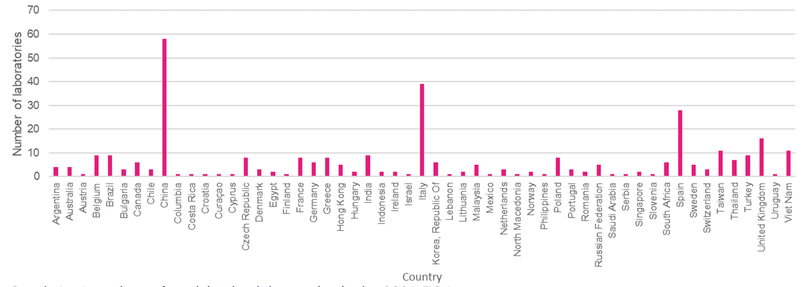
Assessment

A panel expert advisors assessed the submitted anonymised reports against peer ratified criteria. Each participating laboratory received an individual report with scores and expert feedback comments along with a Summary EQA report summarising the expected results and EQA findings.

Results

Participation

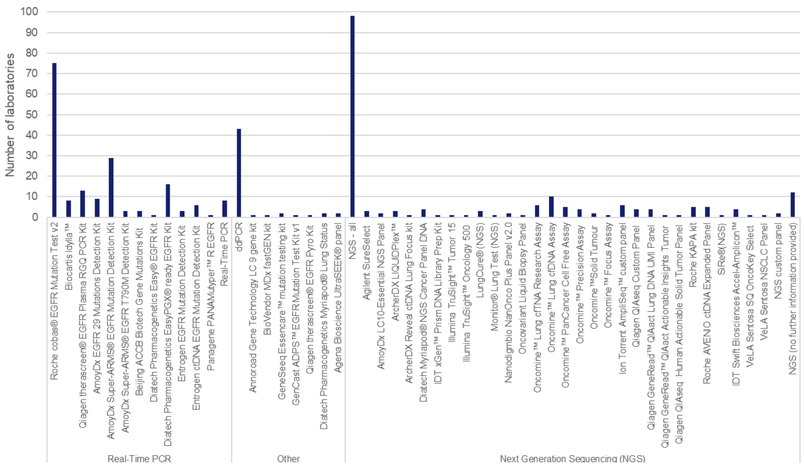
EQA has been delivered to over 250 laboratories (258 in 2020; 292 in 2021) from 55 different countries (see Graph 1).



Graph 1 – Locations of participating laboratories in the 2021 EQA

Participant methodology

- There are >60 different methods used with RT-PCR being the common (see Graph 2), in particular the Roche cobas® *EGFR* Mutation test v2 assay. The second most frequently used method was NGS with many alternative panels utilised. The most common was the Oncomine™ lung cfDNA assay.
- Many laboratories use kits not suitable for cfDNA testing e.g. Qiagen *EGFR* therascreen Pyro kit, Ion Ampliseq panels, Illumina TruSight Tumour 15.



Graph 2 – Methodologies performed by participating laboratories in the 2021 EQA

Results

EQA case	Genotypes	Clinical case/reason for plasma testing
2020 1	No *EGFR* pathogenic variants	Metastatic lung adenocarcinoma with an *EGFR* mutation c.2573T>G p.(Leu858Arg). Received first line treatment with an *EGFR*-TKI. Now progressed.
2020 2	*EGFR* variant detected • c.2155G>A p.(Gly719Ser) at 5% VAF	Metastatic lung adenocarcinoma. Testing of tissue failed. Testing of plasma has been requested.
2020 3	Two *EGFR* variants detected • c.2236_2250del p.(Glu746_Ala750del) at 2% VAF • c.2369C>T p.(Thr790Met) at 1% VAF	Diagnosed with *EGFR*-mutant metastatic lung adenocarcinoma. Confirmed deletion in exon 19 of *EGFR* so prescribed *EGFR*-TKI. After six months radiological progression of primary tumour, metastatic lesions are stable.
2021 1	*KRAS* variant detected • c.34G>T p.(Gly12Cys) at 6% VAF	Metastatic lung adenocarcinoma. Testing of tissue failed. Testing of plasma has been requested.
2021 2	*EGFR* variant detected • c. 2573T>G p.(Leu858Arg) at 6% VAF	EBUS sample demonstrated the presence of a TTF1 positive malignancy but insufficient material available for molecular testing.
2021 3	Two *EGFR* variants detected c.2236_2250del p.(Glu746_Ala750del) at 4% VAF • c.2369C>T p.(Thr790Met) at 2% VAF	*EGFR*-mutant metastatic lung adenocarcinoma (deletion in exon 19). Prescribed first generation TKIs. After one year radiological progression.

Key: VAF = variant allele frequency

Gene references: *EGFR* NM_005228.5 ; *KRAS* NM_004985.5

Table 1 – Summary of EQA clinical case scenarios and validated genotypes

Genotyping accuracy

The standard of genotyping is variable with incorrect variants reported, including false positives/negatives although improvement is observed with continued EQA participation (see Table 2). It was observed that in the 2020 EQA low level variants (≤2%) were not reported in 42% of laboratories due to assay limit of detection.

	2020	2021
Critical genotyping errors	57 laboratories (22%)	31 laboratories (11%)

Table 2 – Number of critical genotyping errors reported

Interpretation and clinical reporting

The content of the reports assessed over both EQA runs indicate a lack of specialist knowledge in interpreting cfDNA test data (see Figure 2). In summary many laboratories:

- Did not demonstrate full understanding of the limitations of cfDNA testing. There are technical limitations in terms of analytical sensitivity/LOD but also biological considerations around the levels of cfDNA in the tested samples.
- Inadequately reported the limitations of the assays used and did not provide useful measurements of the limits of detection.
- Over-interpreted a “no actionable variant detected” result, advising that the absence of an actionable *EGFR* variant indicated that the patient would be unlikely to respond to EGFR-TKIs.

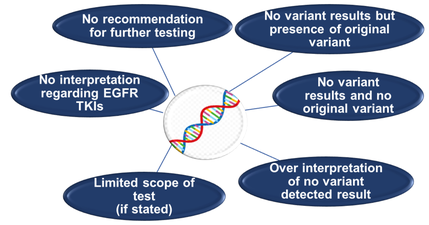


Figure 2 – Common issues observed in the clinical cfDNA EQA reports

Conclusion

EQA detects genotyping errors and highlights the limitations of testing approaches with the aim to improve cfDNA somatic variant testing to aid cancer management. EQA also identifies reporting formats where there is poor interpretation of the cfDNA test result which could be misinterpreted and may cause patient harm. Continued EQA participation demonstrates an improvement in cfDNA testing.

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