Expert Opinion Recommendations for External Quality Assessment for Liquid Biopsy testing from the European Liquid Biopsy Society ctDNA Workshop

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Introduction

External Quality Assurance (EQA) / Proficiency testing (PT) enables independent measurement of the standard of laboratory testing and reporting. The purpose of EQA/PT is to assess the quality of the testing service and to promote good practice through education to ensure safe cancer patient care. It also provides education to aid improvements of services offered by molecular testing laboratories through sharing of good practice.

The increasing number of clinically relevant somatic genetic events that can be detected in plasma-derived circulating cell-free DNA (cfDNA) demonstrates the need for EQA to continually review the scope of its assessments and include key elements for circulating tumour DNA (ctDNA) testing.

Hence the European Liquid Biopsy Society (ELBS) ctDNA working group has produced recommendations to address the current EQA requirements for cfDNA testing based on consensus opinion generated from an interdisciplinary workshop.

Method

A pre-workshop survey collected opinions on the scope of EQAs for clinical cfDNA testing. An in-person 2-day workshop with key opinion leaders enabled discussions to determine consensus opinion and a set of recommendations for EQA in this field.

The workshop was attended by 44 experts including oncologists, pathologists, scientists, clinical chemists, EQA providers, reference material manufacturers, and an In-vitro Diagnostics Regulation (IVDR) specialist. Draft recommendations to address the reference material and EQA needs were formulated based on the collective input, which were reviewed and approved by the poster authors.

Conclusion

Recommendations were formulated for EQA/PT assessments in the current climate with the acknowledgment that they will evolve over time as clinical services develop. EQA providers must ensure that their assessments address the key elements of cfDNA testing as well as the associated reporting requirements.

Acknowledgements: The authors would like to thank all participants of the workshop.
Funding: This study was co-financed by the EU project CAN.HEAL, grant n.101080009 (SAJ, PG).

Results

The following recommendations were agreed by the key opinion leaders.

Scope of variants in EQAs for ctDNA testing:

- Single nucleotide variants (SNVs), somatic copy number variants (CNVs) and fusions for which European Medicines Agency (EMA) approved drugs are available should be included in the assessment.
- The agreed minimum set of targets to be included in EQAs for non-small cell lung cancer (NSCLC) and colorectal cancer (CRC) are listed in Table 1.
- It was recognised that many more tumour types were being tested by NGS panels, therefore a pan-cancer target list was also determined.
- Depending upon the clinical setting, the levels of variants present in the liquid biopsy sample can vary, therefore samples with differing variant allelic frequencies must be utilised.
- The handling of results when no variant has been detected is just as important as detecting variants. EQA/PT must include samples with no actionable variants to ensure the detection of false positive results.
- EQA providers must continually review the scope of the assessments to include new targets when clinical utility has been evidenced.

Table 1 – Minimum set of targets to be assessed

EQA tumour type	NSCLC	CRC	Pan-cancer
Essential	***EGFR*** (including but not limited to): deletions in exon 19, codons 858, 790, 719, 797, insertions in exon 20. ***KRAS*** (codons 12 + 13), Fusions in ***ALK***, ***BRAF, RET*** and ***ROS1***.	***KRAS*** (codons 12, 13, 61, 117 and 146), ***NRAS*** (codons 12, 13, 61, 117 and 146) ***BRAF*** (codon 600)	All essential targets listed for NSCLC and CRC and the following: ***PIK3CA, ESR1, BRCA1, BRCA2, TP53, KIT, PDGFRA, FGFR1, FGFR2, FGFR3***, and ***ERBB2***. *Fusions:* ***FGFR2, FGFR3, NTRK1, NTRK2, NTRK3*** *and* ***MET*** **exon 14 skipping.**
Optional	***BRAF, TP53, KEAP1, STK11, PIK3CA***, ***MET*** **exon 14 skipping**, and CNVs in ***ERBB2***.		***ATM, PALB2 IDH1, IDH2, ATK1, CDK3*** and ***TERT.***

Notes: Reference genes as follows; *BRAF* – NM_004333.6, *EGFR* – NM_005228.5, *KRAS* – NM_004985, *NRAS* – NM_002524.5, *MET* - NM_001127500.3

Reporting of EQA results

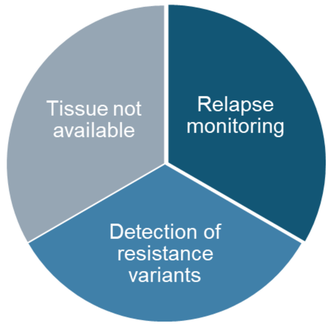
- The reporting of results should be specific to the complexities of the ctDNA scenario.
- As a minimum the following should be stated in a clinical ctDNA testing report:

- ✓ **Correct result including variant allelic frequency (VAF)**
- ✓ **Appropriate interpretation of the results in the context of the clinical scenario**
- ✓ **Details of the test performed and associated limitations**
- ✓ **Further testing recommendations, if appropriate**
- ✓ **Compliance to national/international clinical reporting standards**

Interpretation of EQA results

- The clinical interpretation of results depends on the tumour type and the clinical question being asked therefore EQAs/PT should include clinical details for each sample so the ability of the laboratory to appropriate interpret and report the ctDNA testing results is assessed.
- In particular, the scenarios listed in Figure 1 should be addressed.

Figure 1 – Clinical scenarios to be incorporated into the assessment



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