IQN PATH: Liquid Biopsy EQA– introduction

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On behalf of the IQN Path cfDNA EQA Group

(a sub-group of the Liquid Biopsy Working Group)

Objectives

Initiate a collaboration EQA providers
to provide an EQA to assess the standard of testing cfDNA in plasma
with the purpose of promoting high quality molecular testing.

AIOM

EMQN
The European Molecular Genetics Quality Network

EQA
European Society of Pathology

gen&tiss

GenQA
GENOMICS QUALITY ASSESSMENT

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Objectives

Initiate a collaboration between EQA providers to provide an EQA to assess the standard of testing cfDNA in plasma with the purpose of promoting high quality molecular testing.

- Assess the ability of laboratories to detect cfDNA mutations in artificial plasma samples using a range of methodologies
- Share findings with participant laboratories and the IQN Path Liquid Biopsy Working Group
- Assess the standard of reporting cfDNA testing results
- Testing for mutations in *EGFR* in lung cancer

Scope of EQA

- Well-designed EQA schemes require the development and validation of distribution materials
- Harmonised between several EQA schemes to increase efficiencies and speed of access to EQA
- Include common and clinically relevant mutations/hot spots
- Challenging samples with low frequency allele mutations present at the limit of detection of the methods used should also be included to reassure laboratories that their testing strategies can meet the clinical need.

Warning – too low too soon!!!!

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Design of EQA

- Registration through all 5 EQA providers, free of charge to participants due to sponsorship of the EQA
- 5 artificial plasma samples (3ml)
- Independent validation process
- Sent out by each individual provider with harmonized documentation
- 8 weeks to report results
- Test samples according to routine practice
- Report findings in usual clinical reports
- Harmonized marking criteria used by all providers
- Appeals process
- Poor performance for genotyping only

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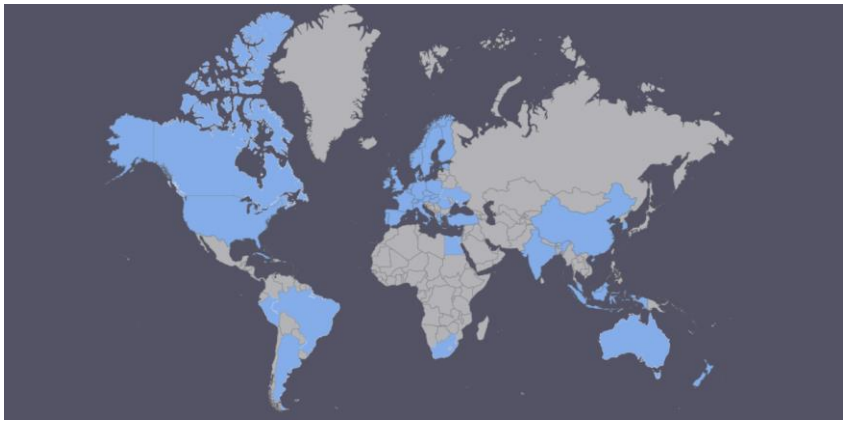
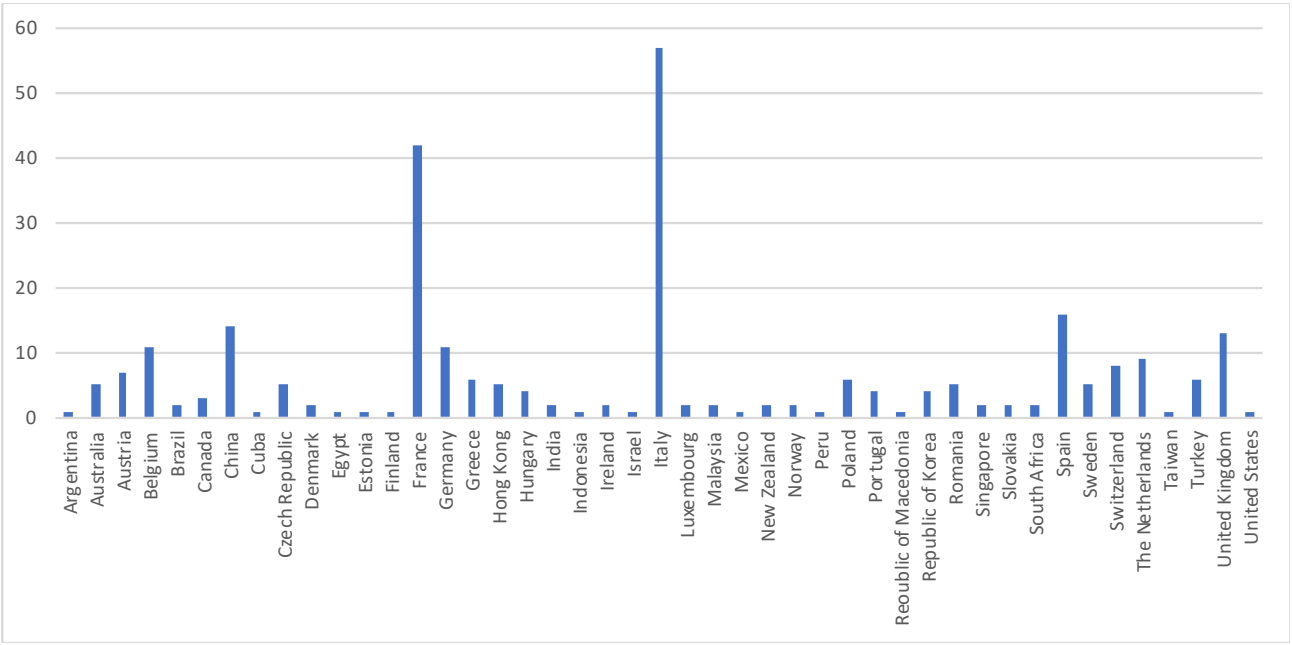
Laboratory participation

- **304 laboratories registered**
- **264 submitted results**
- **45 countries worldwide**





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Samples used for EQA

Sample	Case	Patient details	Reason for referral	Genotype
IQN Path Sample 2018 – A	1	Elena NOVELLO (dob 02/05/1956) Female	Never smoker patient, diagnosed with metastatic lung adenocarcinoma at age 62. EGFR testing performed on the patient's tumour biopsy specimen failed. Testing for EGFR gene mutations on the patient's plasma sample has been requested.	c.2236_2250del p.(Glu746_Ala750del) (1.3% allelic fraction)
IQN Path Sample 2018 – B	2	Sara CIMINO (dob 10/11/1937) Female	Patient with metastatic lung adenocarcinoma diagnosed at age 80. After resection, tumour tissue was analysed and no EGFR variant was detected. EGFR gene testing has been requested on the patient's plasma sample.	No mutations detected within regions tested
IQN Path Sample 2018 – C	3	Ferdinand GARCIA (dob 18/08/1947) Male	Patient with metastatic lung adenocarcinoma, diagnosed at age 68. Patient received first line 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.	c.2369C>T p.(Thr790Met) (5.1% allelic fraction) and c.2573T>G p.(Leu858Arg) (4.7% allelic fraction)
IQN Path Sample 2018 – D	4	David CLARKE (dob 03/10/1962) Male	Patient diagnosed with metastatic lung adenocarcinoma at age 55. The patient was found to have an EGFR mutation and received first line treatment with an EGFR-TKI. At progression of the disease on TKI, the patient had a tissue biopsy but no tumour cells were present. EGFR gene testing has been requested on the patient's plasma sample	c.2236_2250del p.(Glu746_Ala75del) (6.2% allelic fraction)
IQN Path Sample 2018 – E	5	Adele HOLMES (dob 29/09/1952) Female	Patient diagnosed with EGFR-mutant metastatic lung adenocarcinoma at age 65. The patient has a radiological progression of their primary tumour wheras the metastatic lesions are stable. Testing for EGFR gene variants on patient's plasma sample has been requested.	c.2369C>T p.(Thr790Met) (0.81% allelic fraction) and c.2573T>G p.(Leu858Arg) (0.49% allelic fraction)

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Assessment

- Harmonized score criteria drafted in consensus
- Total of 2 points awarded for Genotyping, Interpretation, Patient Identifiers and Clerical Accuracy
- Expert advisors assessed reports for individual EQA providers
- Harmonized assessment meeting for EQA providers
- Changes to marking criteria for case 5

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Marking criteria -Genotyping

Genotyping accuracy → 2.0 marks

Criterion	Deduction
Correct result reported (method enables mutation characterisation AND correct HGVS nomenclature used)	0
Correct result reported (method does not enable mutation characterisation)	0
Correct result reported within the limitations of the test performed	0
False positive result in any gene reported (critical genotyping error)	**2**
False negative result reported (mutation is present below limit of detection or reporting cut-off of assay)	0
False negative result reported (not known if mutation is present below limit of detection or reporting cut-off of assay: critical genotyping error)	2
Incorrect mutation reported (critical genotyping error)	**2**
Only 1 mutation reported reported (for cases with 2 mutations and limit of detection or reporting cut off below variant frequency; critical genotyping error)	**2**
Mutation described incorrectly e.g. incorrect deletion reported at nucleotide or amino acid level (non-critical genotyping error)	0.5
No (or incorrect) HGVS nomenclature used	0.5
Minor HGVS error	0
Technical failure (see comments)	1
Mutation reported at protein level not nucleic acid level	0.5
Method performed is able to characterise the mutation but the characterised result has not been reported	0.5
SNP reported but not identified as SNP	0.5

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Marking criteria -Genotyping

Case 5 – challenging sample – adjusted marking criteria

Score criteria genotyping	Point deduction
Both mutations detected	0
c.2369C>T p.(Thr790Met) only detected	0
c.2573T>G p.(Leu858Arg) only detected and LOD for c.2369C>T p.(Thr790Met) < VAF	1
c.2573T>G p.(Leu858Arg) only detected and LOD for c.2369C>T p.(Thr790Met) > VAF	0
No mutations detected and LOD for both < VAF	1
No mutations detected and LOD for both > VAF	0

Appeals process

- Laboratories given 3 weeks to appeal scores
- 14 (5%) of laboratories submitted appeals

  - 4 laboratory appeals were upheld and the marks returned
  - 1 laboratory appeal was partially upheld and a proportion of the marks returned
  - 1 laboratory appeal was upheld but this did not result in a change to the score
  - 8 laboratory appeals were rejected

- Final report produced by individual EQA providers following appeals process
- Combined report to be distributed to all participating laboratories

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