

Global Delivery of External Quality Assessment for Lung Cancer Liquid Biopsy Testing

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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.



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- 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

Scope of EQA

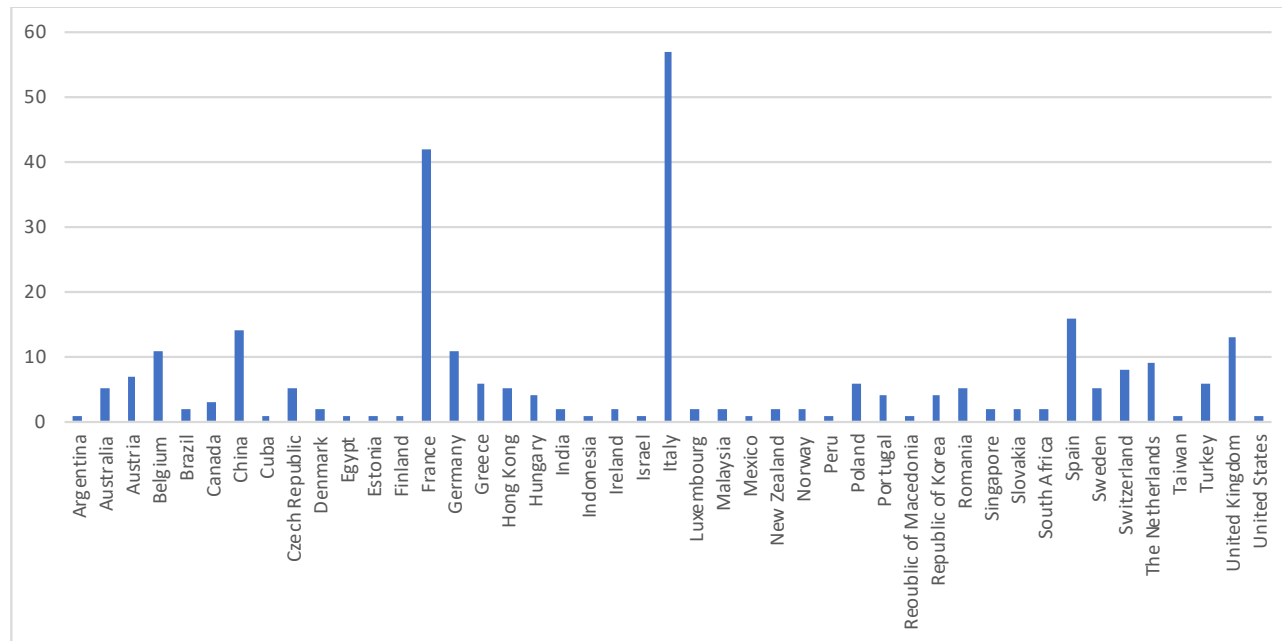
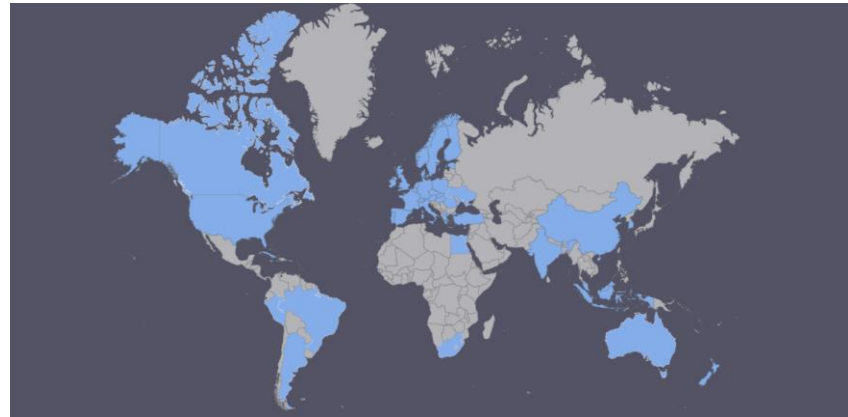
- Well-designed EQA schemes require the development and validation of distribution materials
- Harmonised between several EQA schemes to increase efficiencies and speed of access to EQA
- Include common and clinically relevant mutations/hot spots
- Challenging samples with low frequency allele mutations 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!!!!

Laboratory participation

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

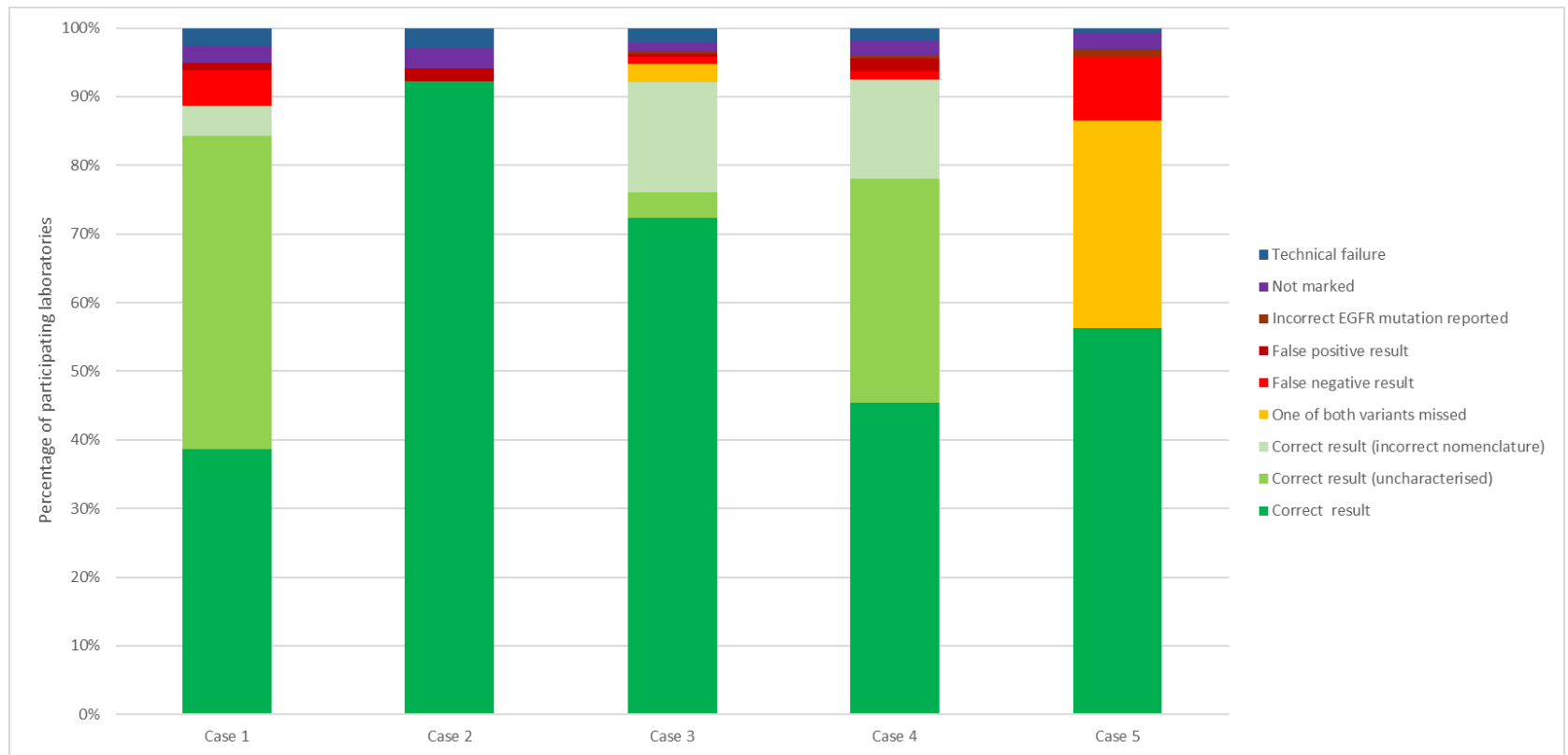


Marking criteria -Genotyping

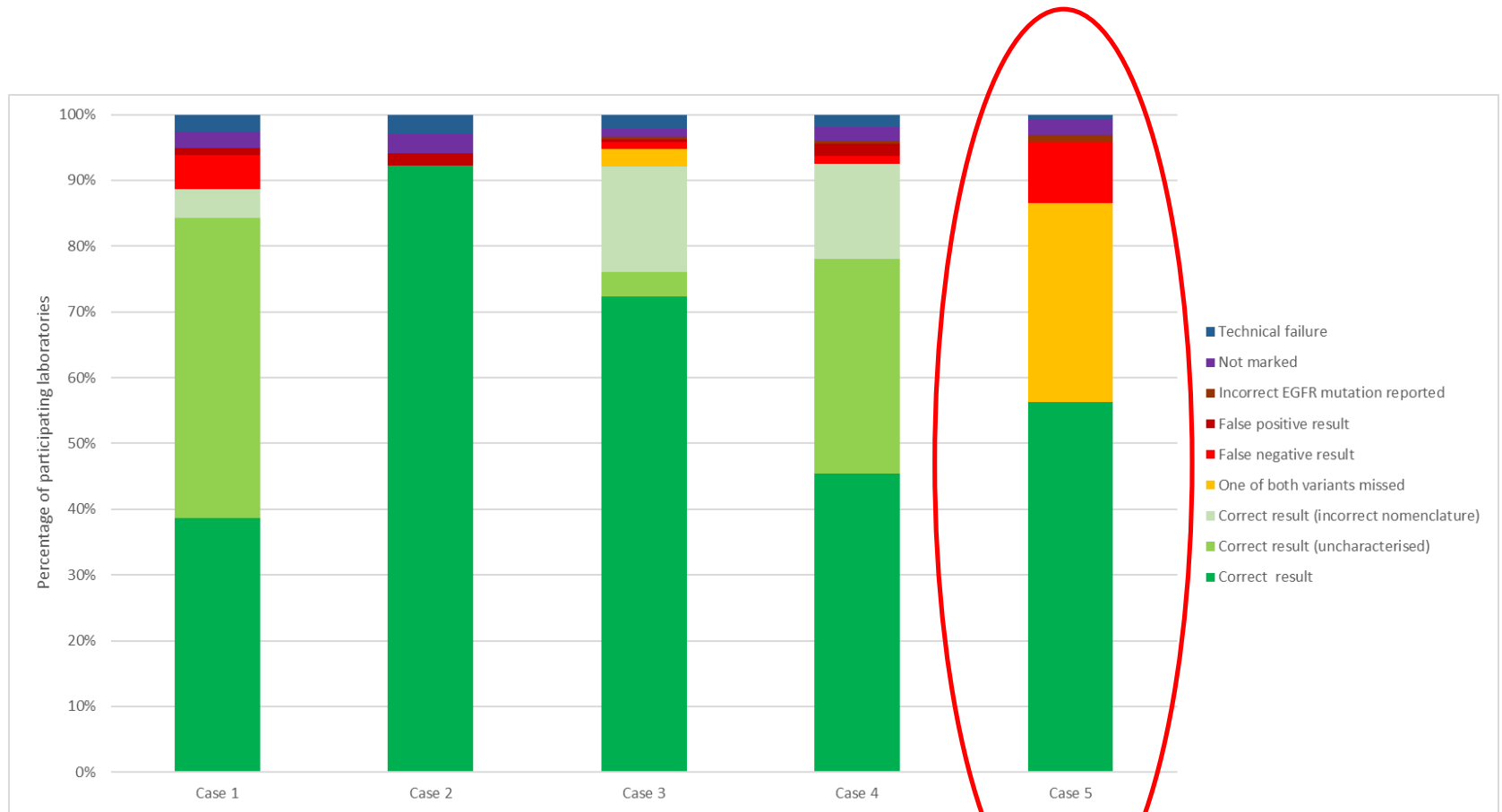
Genotyping accuracy → 2.0 marks

Result reported	Deduction
Correct result	No deduction
Correct results and method does not allow the characterisation of the exact variant change	No deduction
False positive result	-2.0 marks
False negative result with no limits of detection stated in report	-2.0 marks
False negative result with allelic frequency > limits of detection stated in report	-2.0 marks
False negative result with allelic frequency \leq limits of detection stated in report	No deduction
Incorrect point mutation reported	-2.0 marks
For cases with two mutations, if only 1 mutation reported	-2.0 marks
Deletion in exon 19 of EGFR incorrectly described	-1.0 mark
Incorrect HGVS nomenclature	-0.5 marks (deducted only once)

Breakdown of the Genotyping scores



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EGFR – Case 5

- ✓ c.2573T>G p.(Leu858Arg) 0.5% **AND**
- ✓ c.2369C>T p.(Thr790Met) 0.8%

- 147/261 (56%) laboratories correctly reported the presence of both these mutations
- Inconsistent detection of both of the mutations
- Validation results also were inconsistent
- Challenging sample
- Genotyping assessed but laboratories not given critical genotyping errors unless incorrect mutations detected



Reporting issues

Limits of detection

Insufficient information on the limitations of the test performed provided

The most frequently omitted issues were:

- A clear assay description including the mutations that had been assessed
- Clarity around the limit of detection of the assay
- Use of copies/ml without percentage of mutation detectable in a wild-type background

Sensitivity of cfDNA testing

Laboratories failed to state that the analysis of a plasma sample is not 100% sensitive and therefore the presence of a mutation may have been missed

Terminology

Terms 'positive/negative' → *It is recommended to use 'mutation detected/mutation not detected'*

General details

Sample type – *plasma not FFPE*

Inappropriate advice

Laboratories failed to interpret the results in the context of the case provided e.g. first line versus progressing

Next Steps



- **Planning for further EQA in 2019-20**
- **Continue with collaboration**
- **Aim to provide for all interested laboratories**

Acknowledgements

- **IQNPath** *Liquid Biopsy Working Group*

- **Validation Laboratories**

- University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands
- CROM, via Ammiraglio Bianco 83013 Mercogliano (AV) Naples, Italy
- Gustave Roussy, Rue Edouard Vaillant 114, 94800 Villejuif, France
- Laboratoire de Biochimie, CHU Hotel Dieu, Quai Monsousu 9, 44000 Nantes, France
- Manchester Centre for Genomic Medicine 6th Floor St Mary's Hospital Oxford Road Manchester M13 9WL United Kingdom

- **The Sponsors**



- **The EQA providers:**

