Summary of IQN Path cfDNA testing in Lung Cancer EQA 2018

Progress to date

Dr Sandi Deans

Director GenQA/UK NEQAS, Edinburgh, UK

On behalf of the IQN Path ctDNA pilot EQA Group

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

Objectives

To provide as a collaboration, an EQA to assess the standard of testing cfDNA in plasma in lung cancer patients with the purpose of promoting high quality molecular testing and reporting.

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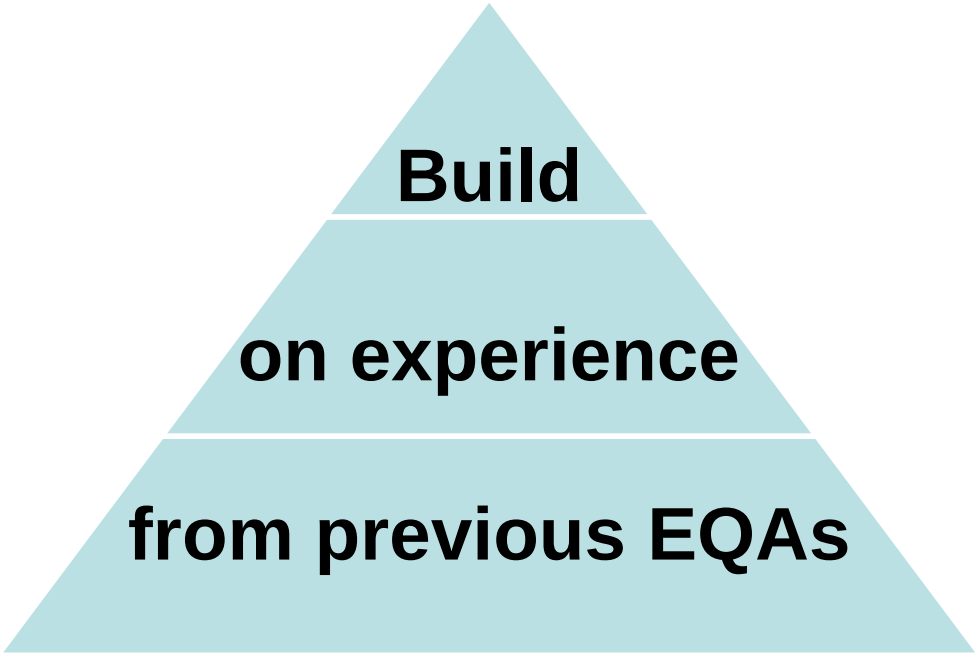
- Assess the ability of laboratories to detect *EGFR* gene mutations at a range of allelic frequencies, in combination with other *EGFR* gene mutations present in manufactured plasma samples using a range of methodologies.
- Share findings with participant laboratories and the IQN Path Liquid Biopsy Working Group.
- Assess the standard of genotyping accuracy and the reporting cfDNA testing results.

Scope of EQA

- Centralised sample sourcing and validation of EQA materials
- Include common and clinically relevant mutations/hot spots
- Include challenging samples with low frequency allele mutations present at the limit of detection of the methods
- Apply performance criteria for genotyping



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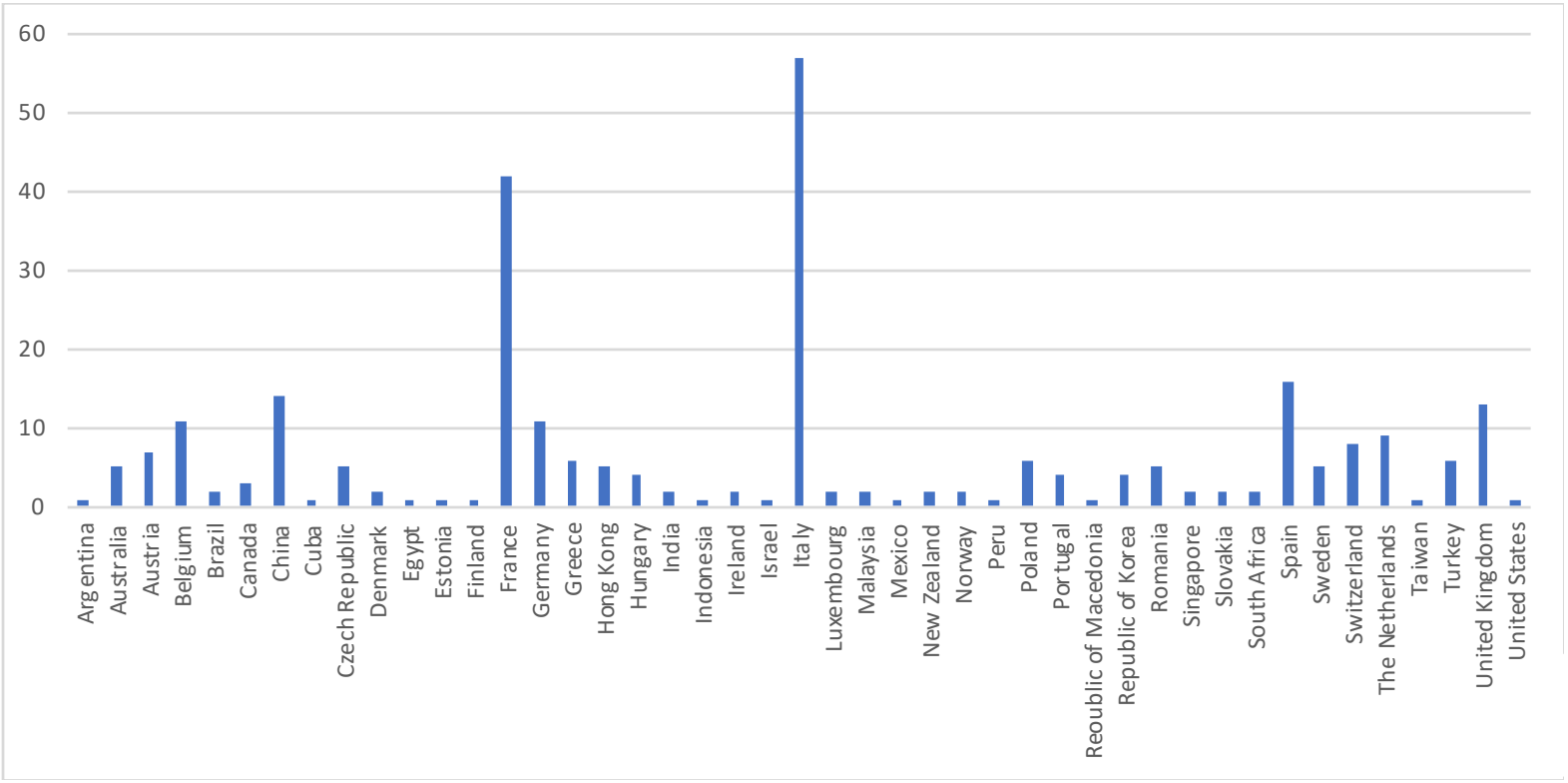
Laboratory participation from 5 EQA Providers

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



Format of pilot EQA

Registration

Sample sourcing & validation

EQA distribution

Results submission

Marking critiera defined

Assessment

Collation of results

Individual EQA Provider Scheme reports plus laboratory reports

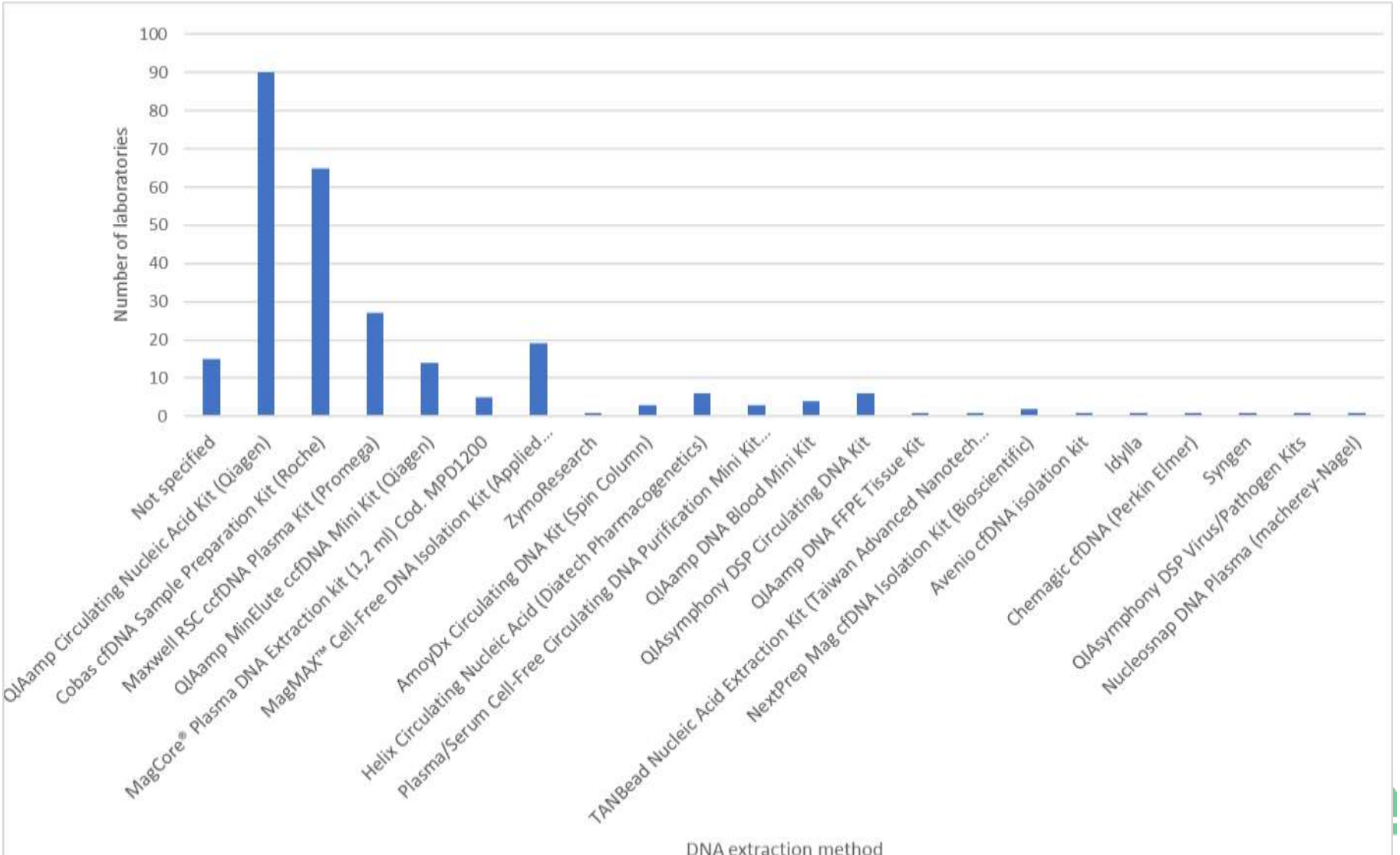
Appeals process

Finalise reports and apply performance critiera for genotyping

DNA extraction methodologies

- Large number of different kits used
- Not all laboratories specified the method performed for DNA extraction

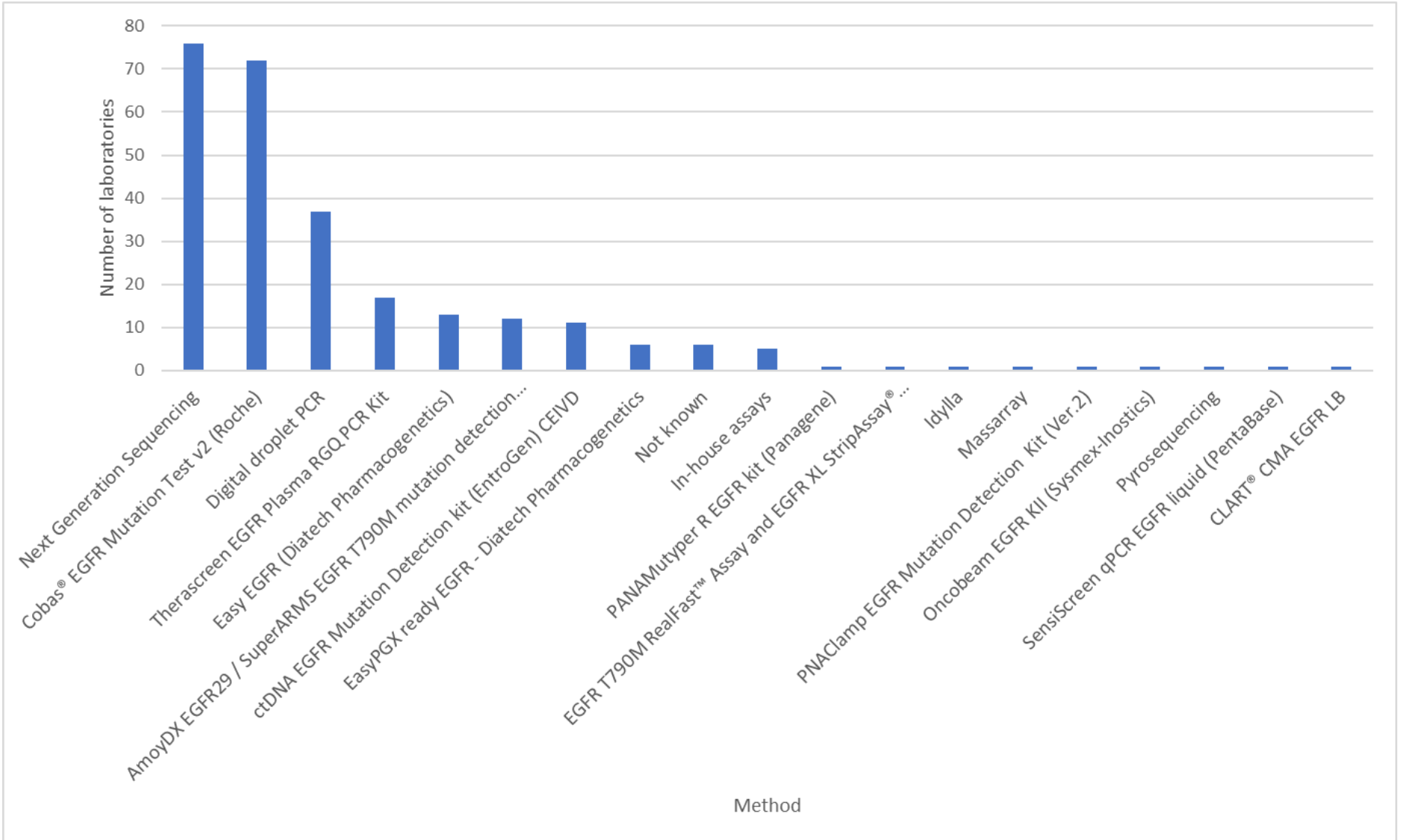
Range of DNA extraction methodologies utilised



Testing methodologies

- Considerable variation in the type of method information provided
- Improvement in the description of NGS test methodologies from previous EQA runs

Range of testing methodologies performed



Marking criteria - Genotyping

Genotyping accuracy assessed out of 2.0 marks

Result reported	Deduction
Correct result	No deduction
Correct results - method does not allow the characterisation of the 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 ≤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)

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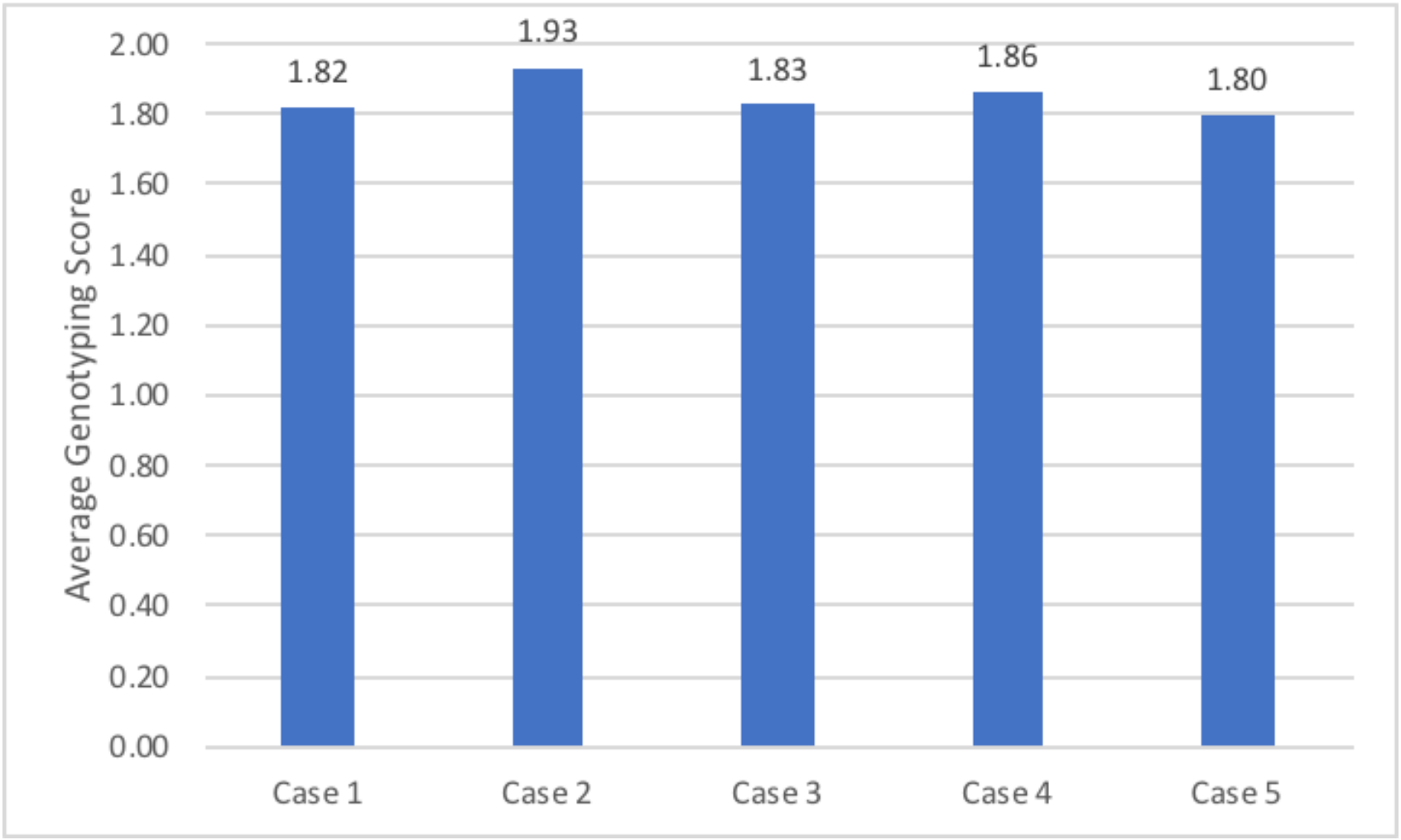
Clinical Cases and expected genotypes

Patient	Reason for referral	Validated genotype
Case 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.	c.2236_2250del p.(Glu746_Ala750del) [1.3% VAF]
Case 2 Sara CIMINO (dob 10/11/1937) Female	Patient with metastatic lung adenocarcinoma. 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
Case 3 Ferdinand GARCIA (dob 18/08/1947) Male	Patient with metastatic lung adenocarcinoma, received first line *EGFR*-TKI treatment and now clinical progression. No tissue sample or cytology specimen due to their poor clinical condition.	c.2369C>T p.(Thr790Met) [5.1% VAF] **and** c.2573T>G p.(Leu858Arg) [4.7% VAF]
Case 4 David CLARKE (dob 03/10/1962) Male	Diagnosed with metastatic lung. Had an *EGFR* mutation and received first line treatment with an EGFR-TKI. At progression 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% VAF]
Case 5 Adele HOLMES (dob 29/09/1952) Female	Patient diagnosed with *EGFR*-mutant metastatic lung adenocarcinoma. Has radiological progression of their primary tumour whereas 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% VAF]

Average genotyping scores – provisional and pre-appeals



Case 5

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

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

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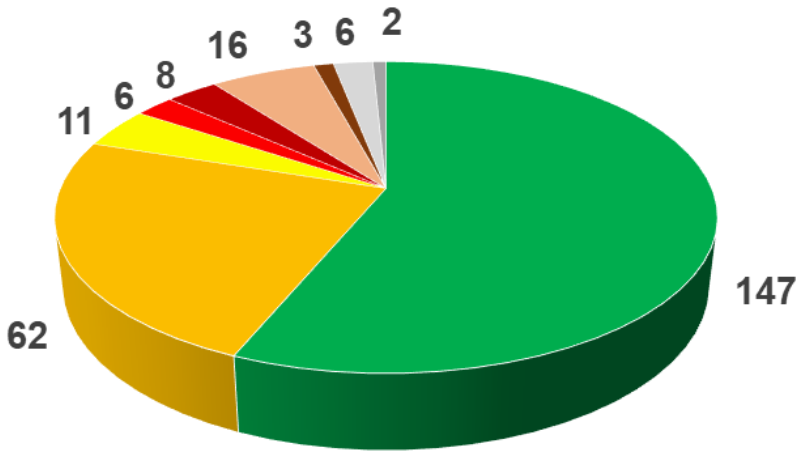

- Number of laboratories reporting correct genotype
- Number of laboratories reporting only T790M
- Number of laboratories reporting only L858R (LOD below that of mutation or unknown)
- Number of laboratories reporting only L858R (LOD above that of mutation)
- Number of laboratories not reporting any mutation present (LOD below that of mutations or unknown)
- Number of laboratories not reporting any mutation present (LOD above that of mutation)
- Number of laboratories reporting false positive results
- Number of laboratories reporting incorrect mutations in EGFR
- Number of failed samples reported
- Not marked/evaluable

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 verus progressing

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Next Steps

- **Release individual laboratory scores and EQA Summary Reports**
- **Facilitate Appeals Process**
- **Finalise scores and apply genotyping performance criteria**
- **Planning for further EQA in 2019-20**
  - **Collaboration of EQA Providers**
  - **Sample provision tender**
  - **Review of targets**

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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**

- **Main Sponsor**

AstraZeneca

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NOVARTIS

- **The EQA providers:**

Aiom EMQN The European Molecular Genetics Quality Network EQA gen&tiss GenQA Genomics Quality Assessment