EQA scheme on liquid biopsy Results

Joined collaboration between 5 EQA providers

AIOM - Italy

EMQN - UK

ESP QA - Belgium

GenQA - UK

Gen&Tiss - France

Kaat Van Casteren

IQNPath

Results

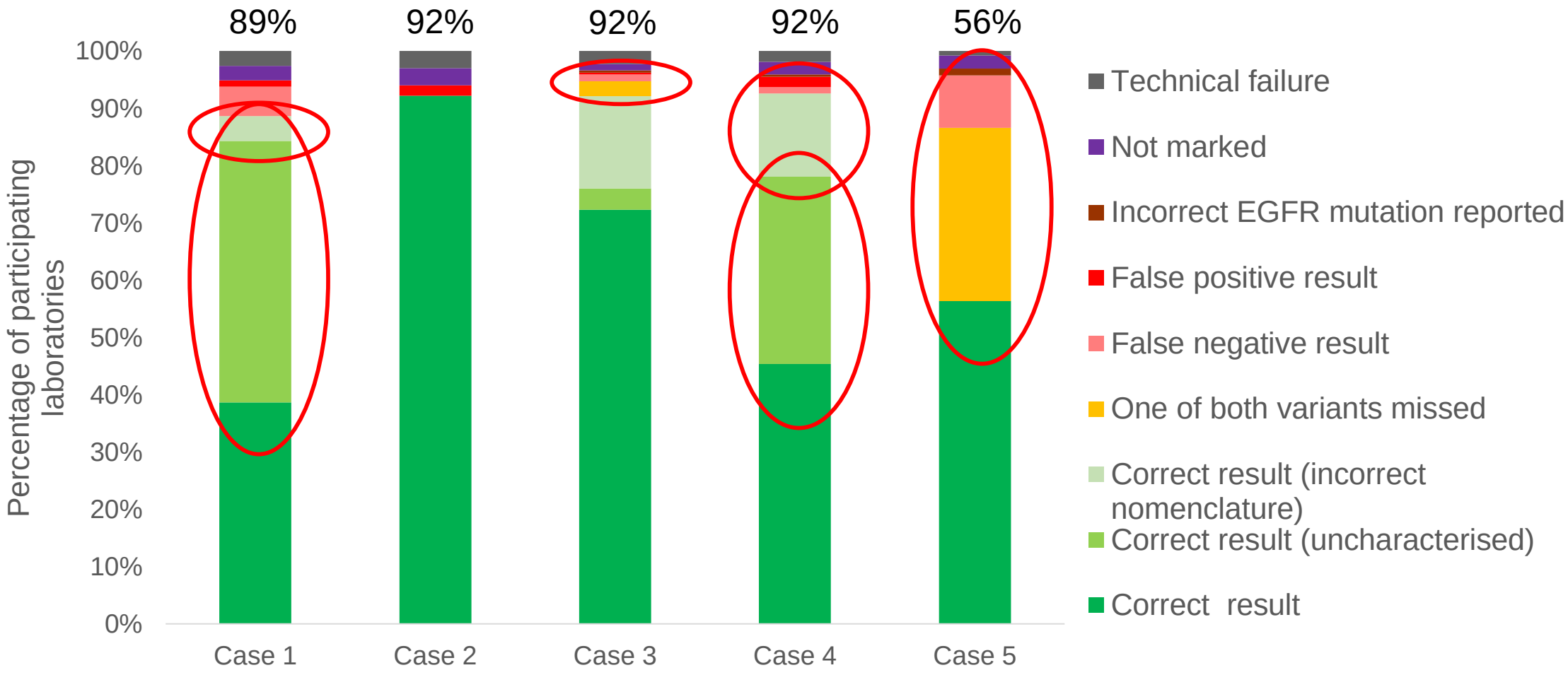
- 264/304 (87%) submitted results
- Genotyping results per case

! 2016 pilot scheme:
1,79/2 for *EGFR* genotyping

Category	Case 1 (exon 19 deletion, 1% VAF)	Case 2 (WT)	Case 3 (L858R & T790M, 5% VAF)	Case 4 (Exon 19 deletion, 5% VAF)	Case 5 (L858R & T790M, 0,5% VAF
Average Genotyping Score (/2)	1.83	1.93	1.82	1.86	1.83
Performance rate (%)*	89%	92%	92%	93%	56%
Error rate (%)*	6%	2%	4%	3%	41%
Technical failures (%)	3%	3%	2%	2%	1%

*Calculated based on the (in)correct identification of all clinically relevant variants in the sample

Results


89%
92%
92%
92%
56%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percentage of participating laboratories
Case 1
Case 2
Case 3
Case 4
Case 5
Technical failure
Not marked
Incorrect EGFR mutation reported
False positive result
False negative result
One of both variants missed
Correct result (incorrect nomenclature)
Correct result (uncharacterised)
Correct result

Results – Case 5

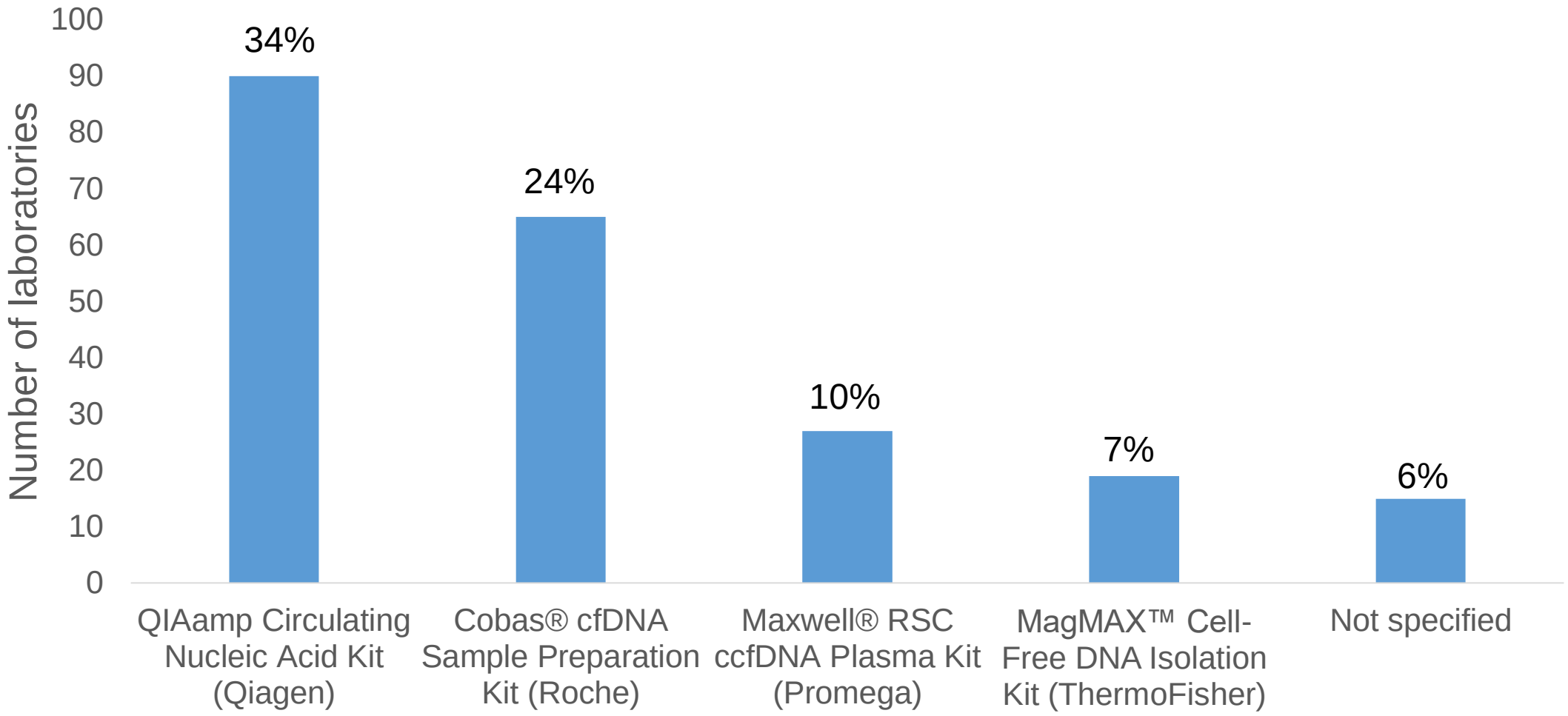
- Challenging case:
  - *EGFR*: c.2573T>G p.(Leu858Arg) at 0,49% VAF
  - *EGFR*: c.2369C>T p.(Thr790Met) at 0,81% VAF
- Adjusted scoring criteria → more lenient
- Overview:

! 2016 pilot scheme: a similar challenging case (VAF 1%) was also excluded from score calculations (61% correct)

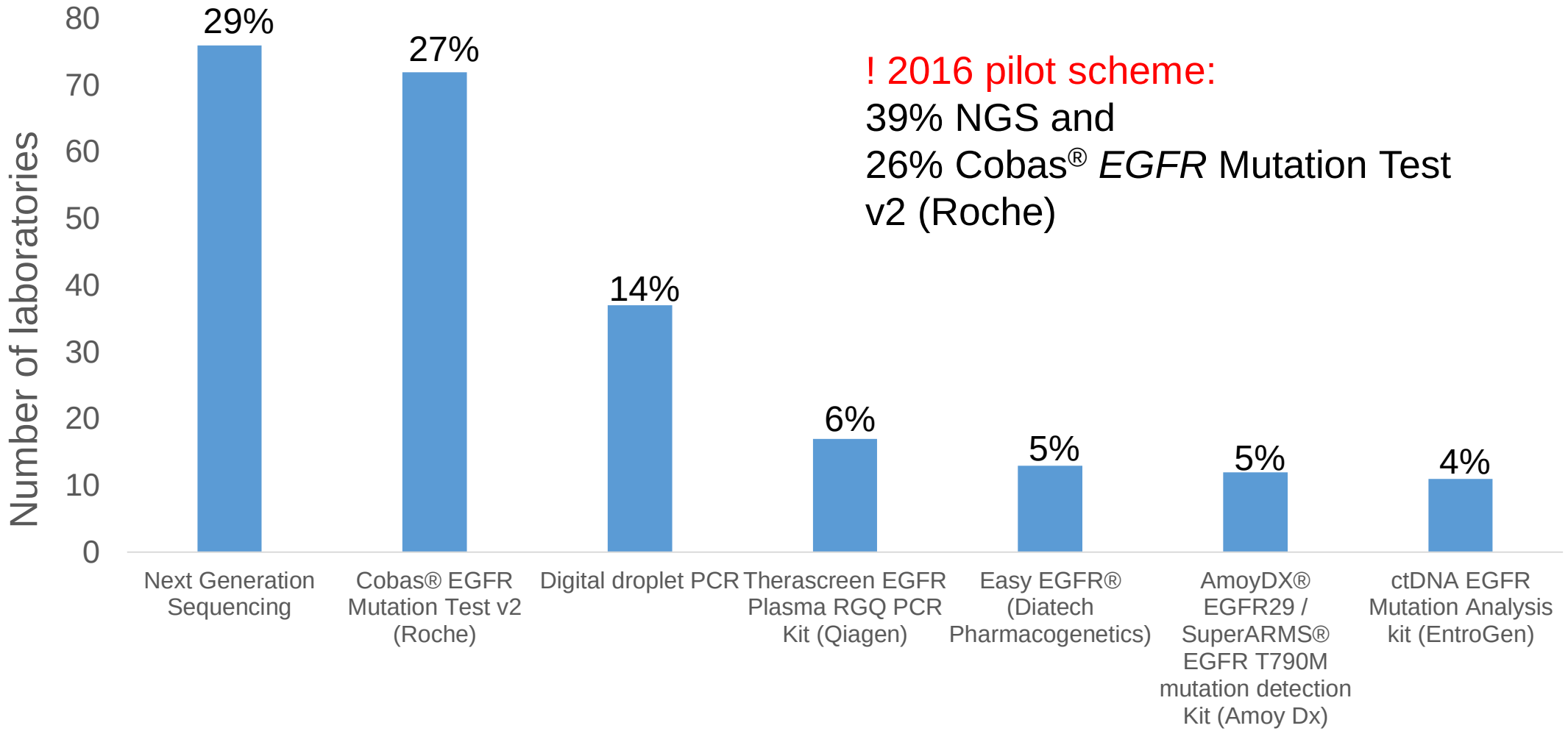
<table>
<tr><th>Missed both variants</th><th colspan="2">Missed one variant</th><th>Incorrect EGFR variant reported</th></tr>
<tr><td rowspan="3">24/264 (9%)</td><td colspan="2">79/264 (33%)</td><td rowspan="3">3/264 (1%)</td></tr>
<tr><td>T790M (VAF 0,81%) missed</td><td>L858R (VAF 0,49%) missed</td></tr>
<tr><td>17/79 (22%)</td><td>62/79 (78%)</td></tr>
</table>

→ Only 56% correct but average score 1,83/2

Results – DNA extraction methods

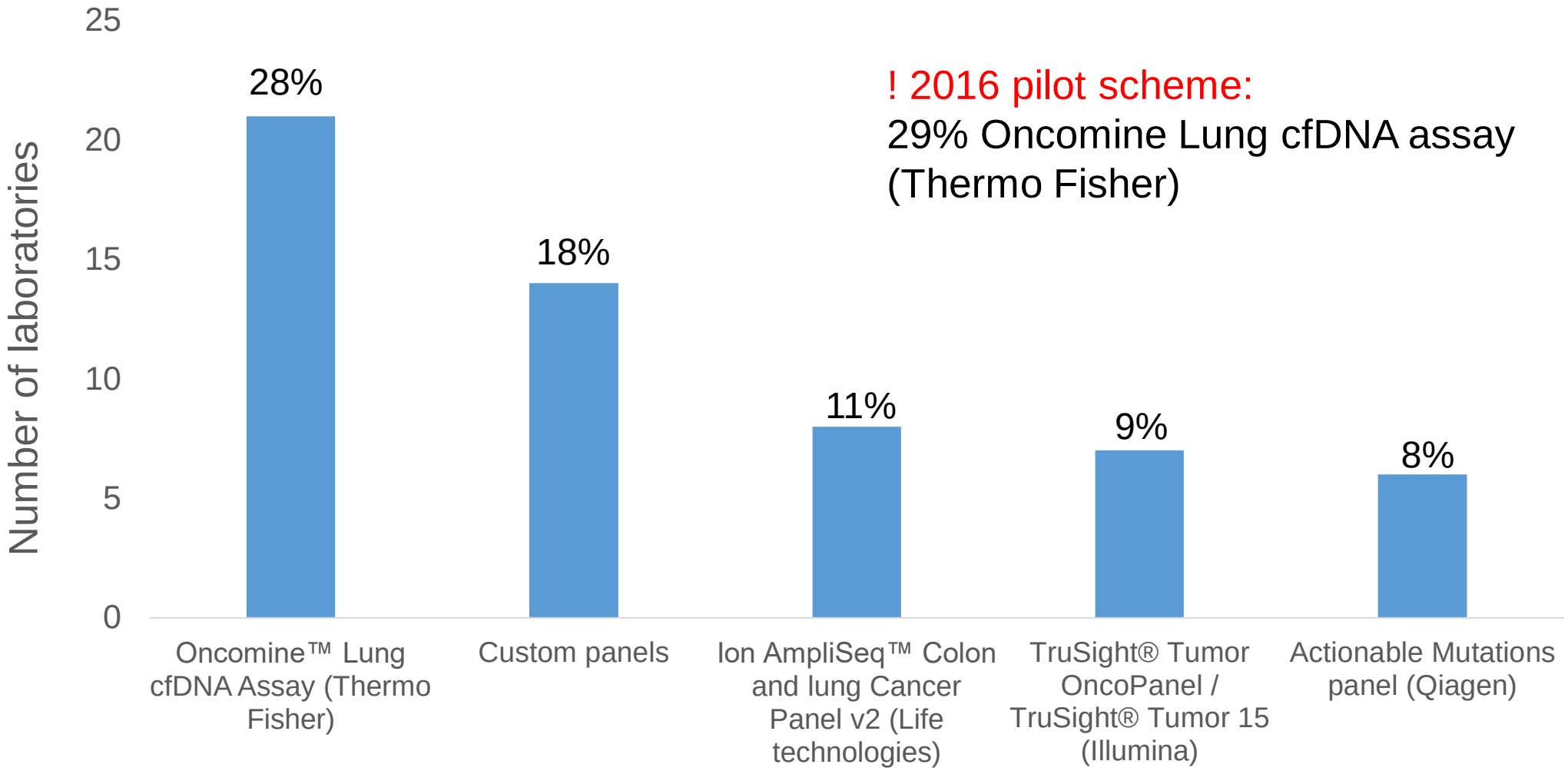


Results – Variant detection methods



! 2016 pilot scheme:
39% NGS and
26% Cobas® *EGFR* Mutation Test v2 (Roche)

Results – NGS methods



! 2016 pilot scheme:
29% Oncomine Lung cfDNA assay (Thermo Fisher)

Results - Reporting

Case 1:
New diagnosis NSCLC

↓

Tissue biopsy failed

↓

Plasma sample: exon 19 deletion (1,3% VAF)

→ **Response to EGFR TKI**

Case 2:
New diagnosis NSCLC

↓

Tumor resection: wild-type

↓

Plasma sample: wild-type

→ **No response to EGFR TKI + statement on sensitivity**

Average Interpretation (/2)*	**1.58**	**1.45**

* AIOM results not included

Results - Reporting

- Important issues - Interpretation:

Case 2:

WT test result at diagnosis

→ No response to EGFR TKI + statement on sensitivity

→ 11% Overinterpretation:
Patient not likely to respond to EGFR TKI*

→ 25% No statement on sensitivity*

* Findings based on ESP QA and GenTiss data only (n=96)

Results - Reporting

Case 1:
New diagnosis NSCLC

↓

Tissue biopsy failed

↓

Plasma sample: exon 19 deletion (1,3% VAF)

→ **Response to EGFR TKI**

Case 2:
New diagnosis NSCLC

↓

Tumor resection: wild-type

↓

Plasma sample: wild-type

→ **No response to EGFR TKI + statement on sensitivity**

Case 3:
Progression under first-line EGFR TKI

↓

No tissue biopsy

↓

T790M (5.1% VAF) and L858R (4.7% VAF)

→ **Response to EGFR TKI (second-line)**

Average Interpretation (/2)*	**1.58**	**1.45**	**1.54**

* AIOM results not included

Results - Reporting

Case 4:
Progression under first-line EGFR TKI

Tissue biopsy failed

Plasma sample: exon 19 deletion (6,2% VAF)

→ **No resistance mutation detected/ no response to second-line EGFR TKI**

Average Interpretation (/2)*	**1.40**

* AIOM results not included

Results - Reporting

- Important issues - Interpretation:

Case 2:

WT test result at diagnosis

→ No response to EGFR TKI + statement on sensitivity

→ 11% Overinterpretation: Patient not likely to respond to EGFR TKI*

→ 25% No statement on sensitivity*

Case 4:

Exon 19 deletion at progression

→ No resistance mutation detected/ no response to second-line EGFR TKI

→ 23% No indication of progressive disease state*

* Findings based on ESP QA and GenTiss data only (n=96)

Results - Reporting

Case 4:
Progression under first-line EGFR TKI

↓

Tissue biopsy failed

↓

Plasma sample: exon 19 deletion (6,2% VAF)

→ No resistance mutation detected/ no response to second-line EGFR TKI

Case 5:
Progression under first-line EGFR TKI

↓

Tissue biopsy not tested

↓

T790M (0,81% VAF) and L858R (0,49% VAF)

→ Response to EGFR TKI (second-line)

Average Interpretation (/2)*	**1.40**	**1.49**

* AIOM results not included

Results - Reporting

- Clerical accuracy - Evaluated criteria:
  - Patient name
  - Date of birth
  - Patient gender
  - Overall layout of report
  - Sample reference number

Category	Case 1 (exon 19 deletion)	Case 2 (WT)	Case 3 (L858R & T790M)	Case 4 (Exon 19 deletion)	Case 5 (L858R & T790M)
Average Clerical Accuracy (/2)*	1.89	1.9	1.9	1.89	1.89

* AIOM results not included

Results

- Reporting – other issues
  - HGVS nomenclature and correct reference sequence not always used[1]
  - Sample type (FFPE instead of plasma)

→No separate template for liquid biopsy reports

  - Testing limitations (copies/mL or %) → standardisation necessary
  - Scope of testing (e.g. only T790M was tested)

→ Not correct in case of first-line treatment

[1] Tack V. et al., 2016. What's in a name? A coordinated approach towards the correct use of a uniform nomenclature to improve patient reports and databases. Hum Mutat 37:570–575.

Acknowledgements

Francesca Fenizia
Nicola Normanno

Melanie Cheetham
Nicola Wolstenholme
Simon Patton

Jenni Fairley
Rachel Butler
Sandi Deans

Cleo Keppens
Ed Schuuring
Elisabeth Dequeker

Marc Denis
Etienne Rouleau

The participating laboratories
The technical and medical experts
The validating laboratories
The assessors