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
Results

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

<table>
<thead>
<tr>
<th>Category</th>
<th>Case 1 (exon 19 deletion, 1% VAF)</th>
<th>Case 2 (WT)</th>
<th>Case 3 (L858R &amp; T790M, 5% VAF)</th>
<th>Case 4 (Exon 19 deletion, 5% VAF)</th>
<th>Case 5 (L858R &amp; T790M, 0.5% VAF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Genotyping Score (/2)</td>
<td>1.83</td>
<td>1.93</td>
<td>1.82</td>
<td>1.86</td>
<td>1.83</td>
</tr>
<tr>
<td>Performance rate (%)*</td>
<td>89%</td>
<td>92%</td>
<td>92%</td>
<td>93%</td>
<td>56%</td>
</tr>
<tr>
<td>Error rate (%)*</td>
<td>6%</td>
<td>2%</td>
<td>4%</td>
<td>3%</td>
<td>41%</td>
</tr>
<tr>
<td>Technical failures (%)</td>
<td>3%</td>
<td>3%</td>
<td>2%</td>
<td>2%</td>
<td>1%</td>
</tr>
</tbody>
</table>

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

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

Percentage of participating laboratories

Case 1: 89%
Case 2: 92%
Case 3: 92%
Case 4: 92%
Case 5: 56%

- 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:

<table>
<thead>
<tr>
<th>Missed both variants</th>
<th>Missed one variant</th>
<th>Incorrect <em>EGFR</em> variant reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>24/264 (9%)</td>
<td>79/264 (33%)</td>
<td></td>
</tr>
<tr>
<td></td>
<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>
<td></td>
</tr>
</tbody>
</table>

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

⇒ Only 56% correct but average score 1,83/2
Results – DNA extraction methods

Number of laboratories

- QIAamp Circulating Nucleic Acid Kit (Qiagen): 34%
- Cobas® cfDNA Sample Preparation Kit (Roche): 24%
- Maxwell® RSC ccfDNA Plasma Kit (Promega): 10%
- MagMAX™ Cell-Free DNA Isolation Kit (ThermoFisher): 7%
- Not specified: 6%
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)*

<table>
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<tr>
<th></th>
<th>1.58</th>
<th>1.45</th>
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</table>

* 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)
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
- Plasma sample: 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

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<th>Average Interpretation (/2)*</th>
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* 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)*

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* AIOM results not included
## Results - Reporting

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

### Clerical Accuracy

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<tbody>
<tr>
<td>Average Clerical Accuracy (/2)*</td>
<td>1.89</td>
<td>1.9</td>
<td>1.9</td>
<td>1.89</td>
<td>1.89</td>
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* 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.
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The assessors