Collaboration between CEQAS and UK NEQAS for Molecular Genetics

NGS, variants and reporting

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UK National External Quality Assessment Services

“helping to ensure clinical laboratory test results are accurate, reliable and comparable wherever they are produced”

➢ Charity organisation
➢ Self funding, non-profit making schemes
➢ 25 specialist centres
➢ 50 years experience providing EQA
➢ ISO 17043 accredited EQA schemes
➢ Participants worldwide
➢ GenQA is a member of this consortium
2019 Delivery of 94 EQAs

- including EQAs with multiple distributions
- including 12 pilot EQAs

78 Countries
Principles of EQA

- Same samples sent to all participating laboratories so benchmarking can be performed
- Independent validation using range of methods so testing agnostic
- Continual performance monitoring
- On-going records of participation and performance so minimal delay in identifying issues

Provides laboratories with an external measurement of the standard of service
Complete genomic testing pathway

Ensuring quality from end to end
Delivering 94 EQAs across 14 specialities

- Haematology
- Newborn screening
- Molecular Genetics core diseases
- Constitutional - postnatal
- Pre-implantation Genetic Testing
- Constitutional - prenatal
- Non-invasive prenatal testing
- Molecular Rapid Aneuploidy testing
- Molecular Pathology
- Next Generation Sequencing
- DNA extraction and quality measurement
- Tumour Assessment (Tissue-i)
- Training/Competency (G-TACT)
- Clinical Genetics Educational
Delivering 94 EQAs across 14 specialities
End to End testing

Pre-test consultation/Referral
Sample
Analysis
Interpretation
Reporting
MDT
Consultation

Pre-test referral

Consultation

Reporting

Sample collection

DNA extraction
DNA quality

DNA quantity

Genotyping accuracy
End to End testing

Pre-test consultation/Referral → Sample → Analysis → Interpretation → Reporting → MDT → Consultation

Pre-test referral

DNA extraction
DNA quality
DNA quantity

Sample collection

Genotyping accuracy

Benign → Likely Benign → Uncertain → Likely Pathogenic → Pathogenic

Reporting → Interpretation → Sample collection → DNA extraction → DNA quality → DNA quantity → Genotyping accuracy

Consultation
Next Generation Sequencing Technical EQA

➢ Germline and Somatic testing
➢ Platform agnostic
➢ Single gene → Panel → WES → WGS
➢ 3 submissions
➢ VCF, BED, FASTQ and BAM files – QC-ed at point of submission
➢ Delivered 3 annual EQAs

Data quality report
(i) Panel of quality metrics
(ii) Benchmarked against same metric from other laboratories

Variant consensus analysis report
Comparison of variants reported against consensus
(i) Concordant variants
(ii) Discordant variants
(iii) Variants not reported
Platforms 2018

2018 EQA
- 260 participants
- 30 countries
Read depth 2018
NGS approach 2018

Bioinformatics
- 69.2% used in-house bioinformatics
- 19.8% outsourced the bioinformatics (2.2% increase compared to 2017 pilot EQA)
- 12% did not state their strategy
Sequencing is (relatively) straightforward

Variant interpretation is not...

- Pathogenic
- Likely pathogenic
- Uncertain significance
- Likely benign
- Benign
**Variant classification**

**SNVs and CNVs**

- 5: Pathogenic (>95%)
- 4: Likely pathogenic (>90%)
- 3: Uncertain significance
- 2: Likely benign (<90%)
- 1: Benign (>95%)

**Variant interpretation is more like....**
BRCA1/BRCA2 variant classification EQA run 1

- A total of **407 individuals** enrolled in run 1 from **59 countries**
- Overall, **271 completed scenarios** were submitted
- Set of 15 variants → Randomised to prevent collusion!
### BRCA1/BRCA2 variant classification EQA run 1

#### 2018 Run 1 - 15 variants to classify

<table>
<thead>
<tr>
<th>Classification</th>
<th>Class</th>
<th>Variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Likely pathogenic</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Uncertain significance</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Likely benign</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Benign</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

#### PARP inhibitor therapy
- PARP inhibitor therapy **recommended**
- PARP inhibitor therapy **NOT recommended**

#### Variants:
- Multiple exon deletion
- Single exon duplication
- Intronic variants
- Deletions/duplications
**Variant Assessment Module**

- List of variants provided
- Clinical details available

<table>
<thead>
<tr>
<th>Test</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGS RASMAPK</td>
<td>29/2/2017</td>
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</table>

**Referral Reason**
- Pulmonary Stenosis

**Referral Type**
- Genetics

**Date of Receipt**
- 27/3/2017

**Priority**
- Normal

**Notes**
Kyle Barnes (dob 15/02/1989) is presenting with pulmonary stenosis and has short stature and a webbed neck. His mother also appears to have features of Noonan syndrome and a local Consultant Clinical Geneticist has requested testing Kyle for Noonan Syndrome.

**PTPN11 (LRG_6141): c.922A>G**

**Gene**
- PTPN11

**Transcript**
- LRG_6141

**cDNA Level**
- c.922A>G

**gDNA Level**
- Chr12(GRCh37) p.112915523A>G

**Protein Variant**
- p.(Asn308Asp)

**Zygosity**
- Heterozygous

**Known Inheritance pattern**
- AD

**Disease segregates in family**
- Mother has similar features, not tested

**Classification**
- Class 4 - Likely to be pathogenic

**Evidence**
Enter the evidence for the above classification here ....

**PARP Inhibitor Treatment**
- Yes
- No
Variant Assessment Module

➢ Or if evidence provided, classify variant
BRCA1/BRCA2 variant classification EQA run 1

Change in clinical management/treatment options
BRCA1/BRCA2 variant classification EQA run 1

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Pre-test referral consultation/Referral
EQA for cfDNA testing for *EGFR* mutations in lung cancer patients

Variability in reporting format

- Submitted reports were anonymously reviewed by a panel of Consultant Oncologists

- **Aims:**
  - Identify the elements which were clear and unambiguous
  - Identify those which were misleading and may cause patient harm if misinterpreted

- The findings were shared with the laboratories to improve the reporting standard of liquid biopsy test results
Case 2

Clinical scenario:  Patient diagnosed with lung adenocarcinoma and treated with EGFR tyrosine kinase inhibitors. Now relapsing and plasma testing has been requested to determine treatment options. The original tumour was found to have a deletion in exon 19 of EGFR.

Plasma testing result: No EGFR mutation detected.
Case 2

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Plasma testing result: No EGFR mutation detected.

Expected interpretation:
➢ Original tumour had a deletion in exon 19 of EGFR.
➢ Plasma sample did not harbour any mutations.
➢ Indicates that there is little or no ctDNA present in the sample and that further testing should be performed.
Case 2 – Oncologists’ Review

Clear and unambiguous statement

CLINICAL COMMENT

No evidence of the common \textit{EGFR} mutations in exons 18 to 21 was found in this cfDNA sample. Given the patient previously tested positive for exon 19 deletion, these results suggest a lack of circulating tumour DNA in the cfDNA of this plasma sample which can occur in up to 20-30\% of patients at progression, particularly those with intrathoracic disease or solitary brain/bone metastases. We therefore recommend obtaining a tissue or cytology sample for \textit{EGFR} mutation analysis if possible.
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❌ Not given in 6/9 reports
❌ Statements about not detecting the primary mutation are “vague”
❌ Not clear enough for clinical use

❌ Not always clear of risk of false negative result
❌ One report - no comment on significance of not finding the primary mutation
  ➡️ “lack of awareness” of ctDNA
Oncologists’ Review - Summary

- Clear reporting of EGFR mutations detected is required
- Avoid vague wording which may lead to misinterpretation
- Variable layout of reports makes finding the result often difficult
- Confirmed or refuted the presence of ctDNA should be mentioned
- Correlation with clinical case is required
- Further testing if appropriate should be stated
Expansion of variant interpretation
Implementation of AMP/ASCO/CAP somatic variant guidelines

Development of NGS technical EQA
Inclusion of other NGS technologies

Extension to somatic NGS technical EQA
Include CNV calling

Implement performance benchmarking
Acknowledgements

- Sample sourcing & validation laboratories
- Scientific advisory groups (SAGs)
- Peer assessors
- GenQA team

Contact us on info@genqa.org