One sample, multiple results.

Farrah Khawaja[1], D. Cregeen[1], L. Slater[2], I. Berry[3], M.P. Lombardi[4], D. O'Sullivan[5], S. Palmer-Smith[6] and Z. Deans[1]

1. GenQA, NHS Lothian, Royal Infirmary of Edinburgh, Edinburgh, EH16 4UX, United Kingdom
2. South East Scotland Genetics Service, Western General Hospital, Edinburgh, United Kingdom
3. South West Genomics Laboratory Hub, North Bristol NHS Trust, Bristol, United Kingdom
4. Department of Human Genetics, Amsterdam UMC, Amsterdam, The Netherlands.
5. North East Scotland Genetics Service, Aberdeen Royal Infirmary, Aberdeen, United Kingdom
6. All Wales Medical Genomics Service, University Hospital of Wales, Cardiff, United Kingdom

GenQA Genomics Quality Assessment
Member of UK NEQAS consortium

P17.025.A

Introduction

Diagnostic genomic testing should be reported accurately, however, external quality assessments (EQAs) demonstrate that laboratories report the same test result in multiple ways which raises concerns about the reliability of the result and may have implications for wider family testing. Four Genomics Quality Assessment (GenQA) EQAs delivered in 2022 included cases where single or multiple exons were deleted/duplicated which highlighted reporting inconsistencies.

Methods

Clinical information and DNA samples were provided for participating laboratories to analyse and report. Participants were expected to report the findings in their laboratory's usual format in the context of the clinical case scenario.

Participants submitted clinical reports that were assessed for genotyping accuracy and interpretation of results by a panel of expert advisors against peer-reviewed marking criteria.

All participants were sent the same DNA samples for each EQA, enabling inter-laboratory comparisons.

The EQA cases were validated independently by at least two laboratories prior to distribution without prior knowledge of the expected result.

Results

2022 Cystic Fibrosis (CF) and *CFTR*-related disorders EQA

Case	Validated result	Number of Participants
1	Homozygous *CFTR* exons 12 to 18 deletion	48

Summary

❖ 25 participants (52%) reported the correct genotype.

❖ 13 participants did not detect the deletion; however, this was correct within the limitations of their test, or they issued failed, inconclusive or interim reports.

❖ One participant reported the "presence of *CFTR* ΔF508" and received a critical genotyping error.

❖ One participant incorrectly stated that the deletion results in a frameshift using the following nomenclature: (Asp529Valfs* 50).

Participants reporting the deletion incorrectly

❖ Six participants correctly reported the presence of a homozygous deletion, however the size of the deletion was incorrect:

- 2 reported a deletion of exons 10 to 16
- 1 reported a deletion located in exon 11 to intron 18
- 1 reported a deletion of exons 11 to 17
- 2 reported a deletion in exons 11 to 18

} participants were advised to use a secondary assay to determine the extent of the deletion.

Nomenclature

❖ Most participants described the deletion according to the exons thought to be involved, for example 'homozygous deletion of *CFTR* exons 12-18'.

❖ Some (24%) also included HGVS[1] nomenclature with only one participant reporting solely with HGVS nomenclature.

❖ The HGVS nomenclature reported by participants differed depending on the testing methodology used.

- Those using the P091 MLPA kit (MRC Holland) to determine the size of the deletion gave consistent nomenclature based on probe locations;

- Those using NGS gave vastly different limits of deletion size, depending on the position of probes or primers used in their test.

❖ Table 1 highlights the range of nomenclature reported, which also included cytogenomic nomenclature (ISCN[2]) for description of the deletion.

❖ Two participants stated that the homozygous deletion included exons 11-16 and no HGVS nomenclature was provided. Although using HGVS is not a requirement when reporting large deletions, the report should clearly state that non-sequential exon numbering of *CFTR* has been used with the reference sequence (NM_000492.3).

Table 1 – HGVS and cytogenomic nomenclature used for reporting *CFTR* exon 12-18 deletions

Nomenclature	Number of Participants
c.(1584+1_1585-1)_(2988+1_2989-1)del	5
c.(1521_1609)_(2941_3047)del	1
c.(1591)_1678)_(3011_3117)del	1
rsa[GRCh37] 7q31.2(117,227,784_117,246,805)x0	1
g.(?_117227793)_(117246807_?)del	1
7q31.2(117227785_117246805) x0	1
c.1584+10_2988+402delinsC	1
c.(1412_1584+25)_(2988+80_2989-52)del)	1

2022 Familial Hypercholesterolaemia (FH) EQA

Case	Validated result	Number of Participants
2	Heterozygous *LDLR* exons 1 to 2 deletion (including promoter)	**21**

Summary

❖ Case 2 included a deletion which participants performing copy number analysis should have been able to detect.

❖ The extent of the two-exon deletion was not always reported correctly.

❖ 15 participants (71%) reported the correct genotype.

❖ Two participants did not detect the variant; however, this was correct within the limitations of their test methodology.

Nomenclature

❖ Three participants did not state that the deletion included the promoter region, which should have been identifiable with the test methodology used.

❖ One participant reported "c.(67+1_68-1)_(190+1_191-1)del", which is incorrect as the deletion includes exon 1.

2022 Epilepsy disorders EQA

Case	Validated result	Number of Participants
2	Heterozygous *TSC2* exons 1-15 deletion	**16**

Summary

❖ 15 participants (94%) reported the correct genotype.

❖ One participant did not detect the deletion using their NGS pipeline, which was assigned a critical genotyping error.

Nomenclature

❖ All 15 participants described the deletion in words:

- 13 stated deletion of exons 1-15;
- One reported genomic deletion of at least 15,764 bp involving the region of coding exons 2-15;
- One stated deletion spans the non-coding 5' region to intron 15.

❖ 11 participants (73%) provided HGVS nomenclature in addition to a written description of the deletion. Table 2 shows the variety of descriptions provided.

Table 2 – Deletion of *TSC2* exons 1-15 described using HGVS nomenclature.

HGVS Nomenclature	Reference Sequence
g.(?_2098180)_(2114285_ 2115566)del g.(?_2098616)_(2114429_2115519)del	NC_000016.9
g.?_2026014_2114553_?del g.(?_2048554)_(2064317_2065534)del g.(?_2097949)_(2114361_2115705) g.(?_2097990) _(2114428_?)del	NC_000016.10
c.(?_-110)_(1599+1_1600-1)del) g.(?_2097639)_(2114498_2115448)del g.(2026012_2114553)del Chr16:g.2089915_2114438del	NM_000548.5
Chr16:2098261-211443	Not stated

2022 Familial Colorectal Cancer and Polyposis (FCRC) EQA

Case	Validated result	Number of Participants
2	*APC* single exon deletion. Exon 2 or 3 depending on numbering system used	**37**

Summary

❖ The deleted exon is exon 2 with traditional numbering i.e., numbering coding exons of NM_000038.6 from 1 to 15 and exon 3 with conventional numbering i.e., numbering all exons in NM_000038.6 from 1 to 16.

❖ 34 participants (92%) reported the correct genotype.

❖ Three participants did not detect the exon deletion despite stating that CNV analysis had been carried out and were therefore assigned a critical genotyping error.

Nomenclature

❖ Four participants reported the deletion as exon 2 using reference sequence NM_000038.6. Without indicating an alternative numbering system, the deleted exon should be referred to as exon 3 using this reference sequence.

Conclusion

In all four EQAs, participants attempted to describe variants using HGVS/ISCN nomenclature and/or exon numbering, with varying degrees of accuracy.

❖ CF and Epilepsy disorders EQAs : HGVS descriptions of multi-exon deletions varied considerably - with up to eight different results reported for CF.

❖ FCRC EQA : a single exon deletion was described differently based on the numbering system used.

❖ FH EQA : the extent of a deletion was not reported consistently.

These results indicate a significant variation in reporting practice for single and multiple exon deletions/duplications which could potentially be confusing for familial/cascade testing if carried out by a different laboratory.

Given the variability seen across participants it is recommended to include the extent of the deletion in terms of exon numbering and to be aware that HGVS and cytogenomic nomenclature will differ substantially depending on the test used.

Including a user-friendly description of the variant **in addition to** the the HGVS/ISCN description and an appropriate reference sequence (and exon numbering system) may therefore be beneficial for clarity e.g., deletion including exon 'a' to 'b' of [gene name] numbering all exons in NM_000XXX.X from x to y.

Acknowledgements: The authors would like to thank the EQA assessors and validation laboratories who have contributed to the delivery of these EQAs.
Conflicts of interest: The authors have no conflicts of interest to declare.

References
1. HGVS Sequence Variant Nomenclature. http://varnomen.hgvs.org/
2. McGowan-Jordan, J., Hastings, R.J., Moore, S. (2020). ISCN 2020: An International System for Human Cytogenomic Nomenclature. DOI: 10.1159/isbn.978-3-318-06867-2. S. Karger, Basel. Also https://iscn.karger.com/

info@genqa.org ● www.GenQA.org ● +44 (0)131 242 6898
farrah.khawaja@genqa.org