Lessons from the 2019 GenQA preimplantation genetic testing external quality assessments for aneuploidies and structural chromosomal abnormalities

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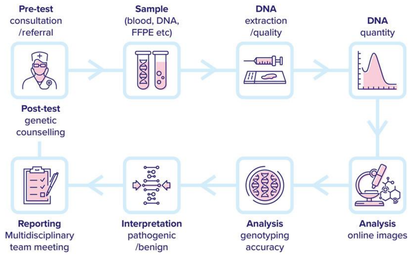
Introduction

Genomic Quality Assessment (GenQA) is an External Quality Assessment (EQA)/Proficiency Testing provider.

We deliver over 100 EQAs to participants in more than 80 countries.

Our EQAs covering all parts of the sample journey.



GenQA has provided preimplantation genetic testing (PGT) EQAs for over 12 years and covers a range of PGT approaches; structural chromosomal rearrangements (PGT-SR), aneuploidy (PGT-A) detection and monogenic disorders (PGT-M) all by different testing methods.

EQA outcomes identify high quality services but also sub-optimal testing and issues with reporting results. This allows laboratories to improve the standard of service for couples undergoing PGT.

The 2019 PGT-A and PGT-SR EQAs used array testing or Next Generation Sequencing (NGS) and identified the over-interpretation of results with regards to mosaicism, and the reporting of abnormalities below the resolution of the assays used.

Mosaicism in PGT

The decision making process for reporting mosaic embryos is guided by

- CoGEN Position Statement (2017)[1]: A threshold of >70% abnormal cells is recommended to class as fully aneuploid for the purpose of clinical practice.
- PGDIS Position Statement (2019)[2]: published works from different groups suggest cut off for aneuploidy assignment to be >80% abnormal cells. This statement now superceded by the PGDIS 2021 statement[3].

It is therefore important to correctly set mosaicism thresholds to allow accurate assessment of whether an embryo is fully aneuploid or mosaic.

References

1. CoGEN Position Statement on Chromosomal Mosaicism Detected in Preimplantation Blastocyst Biopsies. (2017). https://ivf-worldwide.com/cogen/general/cogen-statement.html.
2. Cram, DS *et al.* (2019). PGDIS Position Statement on the Transfer of Mosaic Embryos 2019. RMBO 39:suppl S1. e1-e4.
3. PGDIS Position Statement on the Transfer of Mosaic Embryos 2021. https://pgdis.org/pgd_position.html.
4. Silva, M *et al.* (2019). European guidelines for constitutional cytogenomic analysis. Eur J Hum Genet 27(1):1-16.
5. Coonen, E *et al.* (2020). ESHRE PGT Consortium good practice recommendations for the detection of structural and numerical chromosomal aberrations. Hum Reprod Open. 29;2020(3):hoaa021.

Materials and Methods

PGT-A and PGT-SR EQAs 2019 – for each EQA, laboratories were provided with three clinical case scenarios and corresponding DNA samples for testing.

The samples were mock embryo samples derived from cell lines and were validated independently by three laboratories.

Participants were required to perform routine analysis to look for aneuploidies (PGT-A) or structural chromosomal rearrangements (PGT-SR) and submit a clinical report.

The genotyping results, clinical interpretation in the context of the case information provided, and clerical accuracy of the reports was assessed by a panel of assessors against peer-reviewed marking criteria and current best practice guidelines[4,5].

A final summary report and bespoke individual laboratory reports (ILRs) were issued to all participants.

Results

	PGT-A	PGT-SR
Number of participants	75	65
Number incorrectly reporting mosaicism	4 (5.3%)	4 (6.2%)
Number reporting abnormalities below platform detection limit	N/A	3 (4.6%)

Laboratories incorrectly reporting mosaicism received a critical analytical error.

Mosaicism

Setting an accurate threshold for mosaicism is important.

In both the 2019 PGT-A and PGT-SR EQAs issues were caused by using a sex mismatch to set the mosaicism threshold (Figure 1). If a female patient is matched against a male control then in the patient there are two X chromosomes and in the control one X chromosome. The change is from 1 to 2 - effectively a 100% change.

For an autosome the change to trisomy (or a gain caused by a rearrangement) is from 2 to 3 – effectively a 67% change.

Therefore the log2 ratio threshold for a full autosomal aneuploidy should be set at 67% of the sex mismatch ratio.

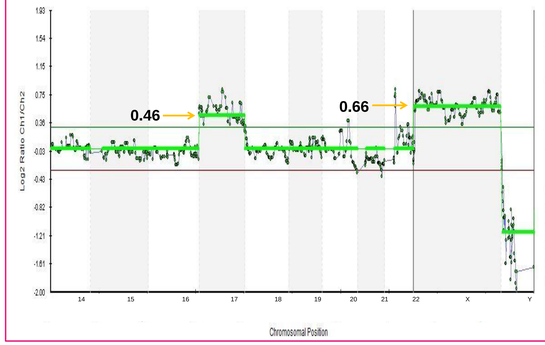


Figure 1: A partial NGS trace showing an example of the log2 ratio for a sex mismatched X chromosome and a full trisomy 17 (not a case from the 2019 EQA).
The Log2 ratio for chromosome 17 is 0.46 and for the sex mismatched X chromosome is 0.66 (0.46/0.66=69%) and therefore the gain of chromosome 17 should be classed as a full trisomy.
Similar results would be obtained for arrays.

Reporting below the resolution

	PGT-A	PGT-SR
Number of participants using NGS	70 (93%)	57 (88%)
Number of participants using array	5 (7%)	7 (12%)

For NGS, the stated practical resolution of the techniques varied between 2 and 30Mb and for array between 5 and 20Mb.

Case 3 of the 2019 PGT-SR EQA involved a recombinant chromosome from a parental inv(16)(q22.1q24.3). The validated result for the embryo was:
arr[GRCh37] 16q22.1q24.3(70603827_89937326)x3
Theoretically the recombinant chromosome would have a 16p loss as well as the 16q gain. However, in this case the breakpoint was very close to the 16p telomere and therefore the loss would not be detectable with the practical resolutions given by the participants.

Three participants incorrectly reported a small terminal loss of 16p, in addition to the expected 16q gain, that would not be detected by their methodology and received critical analytical errors.

The EQA recommendations were that if the abnormality is below the reporting resolution of the platform used it is inappropriate to report the abnormality as this could lead to over-interpretation of the result. Recommendation of further high resolution testing or FISH could be recommended.

A panel of expert advisors reviewed and discussed the issues raised by the EQA and provided feedback to individual participants and offered constructive advice on how they could be addressed.

A summary of the issues identified in these EQAs was also given in an educational ‘Focus on GenQA EQAs for Preimplantation Genetic Testing (PGT)’ webinar in September 2020 (https://www.youtube.com/watch?v=9QIQg6Hkh8c).

Conclusion

It is clear from the results of the 2019 EQA, and indeed more recent EQAs, that these issues remain a problem. It is important that laboratories are aware of the correct method for setting mosaicism thresholds and don’t just rely on pre-set software calling.

It is equally important that the laboratories review the practical resolution of the technique they are performing and optimise the method to give the best detection resolution possible. It is apparent from the ranges of practical resolutions given by the participants that there are major differences in the pipelines used for NGS. It is important to make sure that the methodology is appropriate for the detection of the abnormalities that are expected. This will ensure that that the imbalances are able to be detected and that they are not over-interpreted.

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- GenQA Preimplantation Genetic Testing (PGT) Special Advisory Group (SAG).

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