Novel issues identified through twelve years of external quality assessment of PGT for monogenic disorders.

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GenQA Genomics Quality Assessment
Member of UK NEQAS consortium

Introduction

GenQA (previously UK NEQAS for Molecular Genetics), has provided external quality assessment (EQA) of preimplantation genetic testing for monogenic disorders (PGT-M) since 2008.

Couples can use this procedure as an alternative to invasive prenatal diagnosis or therapeutic termination of pregnancy if they are at risk of passing on a genetic condition to their offspring.

Reliable and accurate testing protocols are typically based on PCR with primers specific for the genetic pathogenic variant plus the addition of linked markers, typically STR markers or SNPs. This direct test plus linkage approach allows testing for the presence or absence of the pathogenic variant(s) as well as contamination and/or amplification issues.

To date, PGT-M for eight disorders including recessive, dominant and X-linked diseases (incorporating triplet repeat disorders) have been provided and has identified common and novel issues.

Material & methods

The EQA format follows a mock PGT case and is provided as two stages to mimic end-to-end testing.

Stage 1: Feasibility testing

❖ Sample type

DNA for a "mock" couple wishing to undergo PGT and an "affected" family member.

❖ Submission requirements

Proforma with genotyping results, providing details of proposed testing strategy including:
- Limitations and risk of misdiagnosis
- Whether strategy deemed sufficient to proceed to stage 2*.

*Laboratories that were unable to perform PGT following stage 1 were withdrawn.

Stage 2: Embryo testing

❖ Sample type

Cells to mimic embryos.
Laboratories chose either#:
- Single cells (blastomere testing) or
- A set of six cells (trophectoderm testing)

❖ Submission requirements

Fully interpretative reports stating genetic suitability for transfer
Proforma detailing haplotyping results.

#From 2008-2013, laboratories provided with single cells only. Since 2014, choice of testing made available.

Format

Thirteen PGT-M EQAs have been provided to date, all but one were sample-based. The 2021 PGT-M EQA was interpretation-only (data not shown).

Assessment

Submissions were assessed anonymously against peer-ratified marking criteria.

Samples were validated for STR analysis (2008 to 2015) and both STR and SNP analysis since 2016, for the disease specified for that EQA run prior to distribution.

Acknowledgements
Eurofins Genoma Laboratories, Italy for support with sample handling and cell validation. Also, the following laboratories for sourcing samples and validation testing: Centre for Medical Genetics, Belgium, Repromeda, Czechia, Guys and St Thomas' NHS Foundation Trust, UK., National and Kapodistrian University of Athens, Greece and Eurofins Genoma Laboratories, Italy.
Acknowledgements also to the Preimplantation Genetic Testing Specialist Advisory Group and assessors.

Results

Participation There has been a 5-fold increase between 2008 and 2021, from 11 to 68 participating laboratories.

Methods STR linkage analysis remains the most used method (59% laboratories in 2020), despite the widespread adoption of SNP-based automated platforms from 2013 onwards.

Material tested Trophectoderm samples were most frequently used for embryo testing; ≥77% laboratories in 2018-2020.

Issues

- Informativity of markers:
  The informativity of markers was often inaccurate and many variable approaches were applied. The EQA stated that laboratories should refer to guidance, in particular the ESHRE recommendations[1] which provide clear advice on assigning informativity and will help to standardise approaches.
- Marker-related information:
  The EQA identified that marker positions may vary according to the human genome build version used which has an impact on the PGT strategy applied. Both distance and position of all markers with respect to the genetic variant should be provided.
- Common assumptions:
  1. Homozygosity - When assessing genotype results for "embryo" samples, many laboratories assumed homozygosity of the alleles. Due to the risk of allele dropout, homozygosity should not be assumed at the single/low cell level.
  2. Genetic distance - Many laboratories assumed genetic distance. For some genes, e.g. *DMD*, recombination occurs more frequently across the gene than would be expected by its length alone. Linkage strategies require selection of mainly intragenic markers, closely flanking the genetic variant that is being tracked.
- Misdiagnosis:
  1. Sex-related: For recessive X-linked disorders, a misdiagnosis will have different consequences depending on the sex of the embryo.
  2. Recombination-related: For challenging genes, e.g. *SMN1*, it can be difficult to locate markers within 1 Mb of the gene, therefore, the location of selected flanking markers can be extended >1Mb for STRs and >2Mb for SNP haplotyping protocols. However, including more distant markers increases the risk of misdiagnosis and this should be addressed when reporting the results.

Performance Issues remain as evidenced in the 2021 EQA with two laboratories (3%) reporting incorrect results for embryo testing and four laboratories (6%) reporting interpretation errors.

Conclusion

Participating in EQA leads to better testing in the long term by identifying areas for improvement. GenQA has been providing PGT-M EQAs for over 12 years. By alternating the disorders and different modes of inheritance, various aspects of testing have been addressed and learning outcomes highlighted to ensure the correct embryo results are reported. Whilst the incidence of genotyping/interpretation errors remains low, they are reported annually, demonstrating the need for continual EQA participation.

References [1]ESHRE PGT Consortium Steering Committee, ESHRE PGT Consortium good practice recommendations for the organization of PGT. Hum Reprod Open May 29;2020(3)

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