Non-invasive prenatal testing EQA – improving the detection of sex chromosome aneuploidies.

Fiona J. Morgan[1], Farrah Khawaja[1], Weronika Gutowska-Ding[2], Simon J. Patton[2], Zandra C. Deans[1]

1. GenQA, NHS Lothian, Royal Infirmary of Edinburgh, Edinburgh, EH16 4UX, United Kingdom,
2. EMQN CIC, Unit 4, Enterprise House, Pencroft Way, Manchester Science Park, Manchester, M15 5SE, United Kingdom

Introduction

External Quality Assessment (EQA) for non-invasive prenatal testing (NIPT) for common aneuploidies has been offered in collaboration between GenQA and EMQN since 2017. The standard of testing for evidence of trisomies 13, 18 and 21 was delivered and later the option for assessment of NIPT for sex chromosome aneuploidies was introduced to reflect change in clinical practice. Initial EQA results following the inclusion of sex chromosome aneuploidies saw an increase in poor performance, indicating the need for improvement in both the testing and reporting of this subset of NIPT cases.

The notable improvement in participant performance observed in subsequent EQA rounds, following educative feedback and effective guidance, shows the importance of EQA participation for non-invasive prenatal testing where the scope is rapidly evolving.

Methods

Samples

- Two plasma samples (patient and/or artificial material) with corresponding clinical cases are provided for each NIPT EQA.
- Maternal plasma was sourced from RAPID Biobank and artificial material from SeraCare/LGC Group. The NIPT result is confirmed ahead of EQA distribution, using either standard prenatal testing such as QF-PCR (patient material) or independent validation by at least two laboratories using different NIPT platforms (artificial material).

Testing

Participants are required to:

- Perform routine NIPT of the samples provided using their standard laboratory protocols.
- Submit their clinical reports for assessment.

Assessments

- EQA submissions are marked against peer-reviewed marking criteria.
- Marked by an established team of expert assessors.
- A critical genotyping error is given where erroneous NIPT results are reported within the remit of the test performed.

EQA outcomes

- Following assessment, tailored performance feedback is provided to participants via an individual laboratory report (ILR).
- Publication of an EQA summary report allows participants to benchmark their performance against other participating laboratories, and provides recommendations for improvement in both testing and reporting of NIPT results.

Figure 1. Marking criteria used to determine critical error (Expected result: high chance XYY)

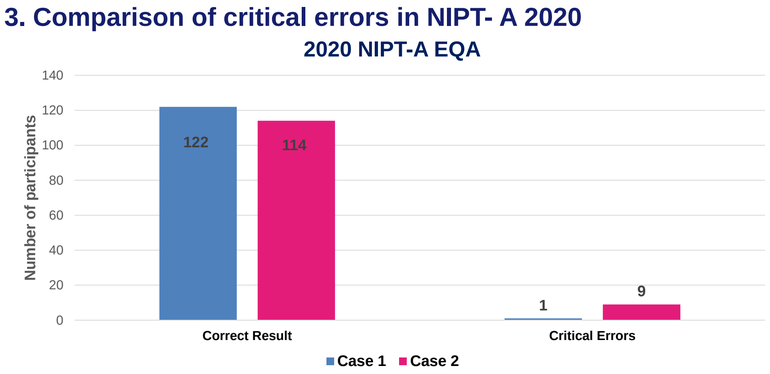
EQA Marking Criteria		
Genotyping	Evidence of XYY, male fetus	2.0 marks
Interpretation	Low risk for trisomy 13, 18 and 21 High risk for sex chromosome aneuploidy XYY	2.0 marks

Results

Accuracy of reporting sex chromosome aneuploidies using NIPT

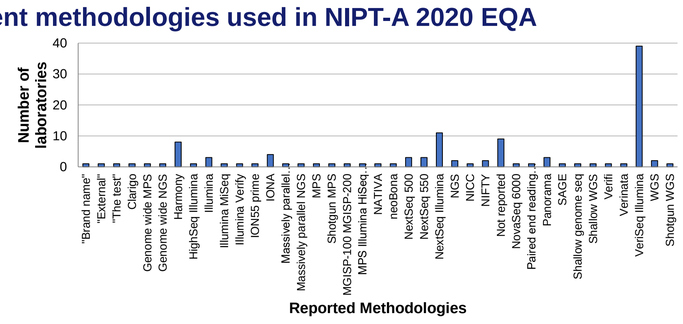
Initial inclusion of an XYY case in the NIPT for common aneuploidies EQA (NIPT-A) in 2020 resulted in an increased rate of critical genotyping errors, when compared to reporting of high chance result for autosomal aneuploidy. (Figure 3) Only one critical genotyping error was given in Case 1, where a participant failed to report the high chance result for trisomy 13 (1/123 participants, 0.8%). This critical error rate increased to 7.3% for case 2 where nine of the 123 participants failed to report the high chance XYY result.

Figure 3. Comparison of critical errors in NIPT- A 2020



Detailed evaluation of the critical genotyping errors reported across this test agnostic EQA, excluded any platform-specific errors. A total of 39 different methodologies were described by the 123 participants of this EQA (Figure 4). Those participants who failed to detect evidence of the XYY result described use of various methodologies including IONA, VeriSeq, next generation sequencing (NGS) and massively parallel sequencing.

Figure 4. Different methodologies used in NIPT-A 2020 EQA



Dynamic Evolution of NIPT EQAs

In line with the rapid evolution of this unique prenatal test, the NIPT for common aneuploidies EQA was updated to include reporting of fetal sex, sex chromosome aneuploidies, and evidence of trisomies for chromosomes 13, 18 and 21. Submissions were assessed based on the reporting option selected. (Figure 2)

Figure 2. Revised EQA reporting options

Reporting Option	Reporting Option Details
1	Aneuploidy testing for chromosomes 13, 18 and 21 only. Fetal sex not reported.
2	Aneuploidy testing for chromosomes 13, 18 and 21. Fetal sex reported.
3	Aneuploidy testing for chromosomes 13, 18, 21, X and Y. Fetal sex reported.

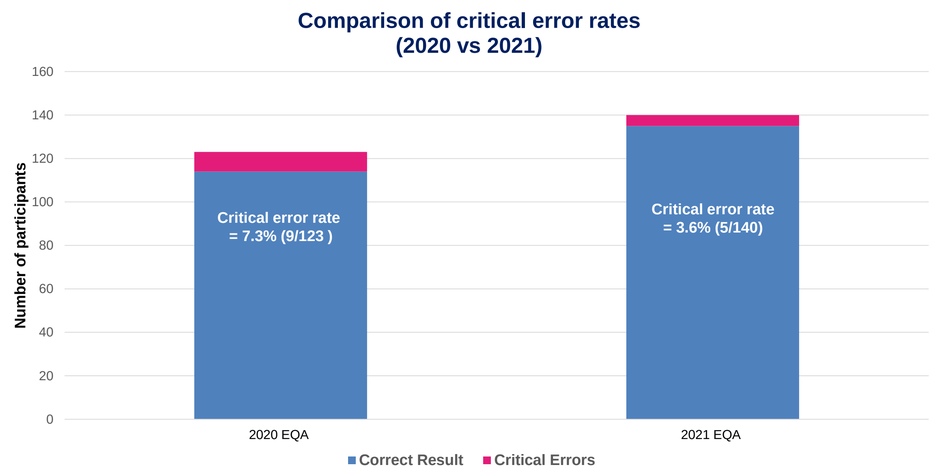
Improved detection of sex chromosome aneuploidy

Further comparison of results when an identical sample was issued in the 2021 EQA (Figure 5), showed increased accuracy in the reporting of a high chance result for sex chromosome aneuploidy. The number of critical genotyping errors fell to 3.6% (5/140 participants), almost half the initial figure in 2020 (7.2%), when the same XYY sample was re-issued in 2021. (Figure 6)

Figure 5. EQA cases provided in 2020 and 2021, both with expected XYY result.

EQA year	Case ID	Patient Details	Gestational Age	Reason for Referral	Expected result
2020	101NIPT20	Julianna FLEURIE 11/11/1991	14+2 weeks	Requested NIPT for common aneuploidies as she did not undergo first trimester ultrasound/combined screening.	Low chance result for trisomy 13, 18 and 21 **High chance result for sex chromosome aneuploidy XYY** MALE FETUS
2021	101NIPT21	Aiko TANAKA 31/12/1980	14+2 weeks	Combined screening risk of 1 in 130 for Trisomy 21.	Low chance result for trisomy 13, 18 and 21 **High chance result for sex chromosome aneuploidy XYY** MALE FETUS

Figure 6. Improved EQA performance



This reduction in critical errors between EQA rounds confirms improved performance as a result of continued EQA participation, highlighting enhanced laboratory performance through educative feedback from EQA providers.

Conclusion

Inclusion of NIPT for sex chromosome aneuploidies re-affirms the importance of EQA as a mechanism to independently measure the standard of laboratory testing, particularly where the scope of current testing expands. Initial results confirm improvement in both accuracy of testing and standard of reporting as a consequence of tailored EQA feedback to participants.

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info@genqa.org • www.GenQA.org • +44 (0)131 242 6898

office@emqn.org • www.emqn.org • +44 (0)161 757 1591