QST*Rplusv2 REDUCES TURNAROUND TIMES AND SAVES MONEY COMPARED TO COMPETITOR PRODUCT

INTRODUCTION

Quantitative fluorescent–polymerase chain reaction (QF-PCR) has been shown to be a reliable and efficient methodology for the rapid diagnosis of the common autosomal and sex chromosome aneuploidies during pregnancy[1]. Using this approach, chromosomes are 'counted' by way of assessment of height ratios or peak area for informative short tandem repeat (STR) markers. Where an STR marker is the same size from both the paternal and maternal contribution, i.e. a homozygous result generating a single peak, this would be considered uninformative. More specifically, loci are tested and chromosome number is inferred from the aggregate result. A trisomy is detected either by the presence of three equal peak ratios (1:1:1), or two peaks with a skewed ratio (2:1).

Most laboratories would consider a result to be accurate and reportable providing at least two markers are informative for the given chromosome. In cases where fewer than two markers are informative for a given chromosome laboratories are required to undertake further testing. Many options may be considered, for example karyotyping or array CGH. However, it is commonplace to perform so called 'reflex' QF-PCR to examine additional STR markers for the chromosome in question. Irrespective of the analysis method chosen this extra evaluation step will increase costs and turnaround times.

Several commercially available QF-PCR options exist to undertake testing, including the Yourgene Health QST*Rplusv2 kit. Yourgene Health also offer a suite of single chromosome 'reflex kits' for laboratories to use in the event of an uninformative result. These kits contain additional chromosome specific markers in order to obtain the necessary number of informative results. A laboratory considering several commercial kits prior to purchase will likely consider the rate of required reflex testing as part of performance evaluation, due to the potential impact on turnaround times and cost. The rate of reflex testing is closely related to the original choice of STR markers for inclusion in the kit, as well as the patient population being tested.

The Molecular Laboratory within the University of Alberta Department of Medical Genetics, Canada, offers a comprehensive diagnostic and clinical service as well as an exceptional cross-functional translational research effort. In an effort to reduce turnaround times and costs associated with delivering a high-throughput rapid prenatal aneuploidy service, the department evaluated the performance of the AneufastTM v2 (MolgentixSL) QF-PCR assay alongside the QST*Rplusv2 kit[1]. Yourgene Health have also been made aware of an assessment of reflex testing rates using the kit by the Genomics Laboratory at the Royal University Hospital of Saskatoon, Canada. The laboratory is a major centre for reproductive and prenatal genetic testing, and the findings of their performance evaluation are also discussed below.

KEY FEATURES AND FINDINGS

- **QST*Rplusv2 required less frequent reflex testing, which translates into shorter turnaround time and cost savings (Table 1).** The Alberta group report that for this sample set the Aneufast™ v2 (MolgentixSL) test required reflex testing at a higher rate across all chromosomes considered than the QST*Rplusv2 assay. This finding is in line with the reported performance metrics from the Royal Hospital in Saskatoon (Table 2)
- **QST*Rplusv2 design enables superior diagnostic performance of sex chromosome aneuploidies.** The Alberta group report that for the sex chromosomes, the difference in the amount of follow-

up testing is greater between the assays as a result of the inclusion in the initial PCR of the TAF9L paralogous marker in the QST*Rplusv2 assay

- **QST*Rplusv2 requires less troubleshooting and is easier to handle in a diagnostic environment.** The presence of dye blobs, particularly from the PET dye and differences in PCR efficiencies between S1 and S2 mixes can be problematic for some samples ran with Aneufast v2.

The Alberta publication reports that to confidently rule out a chromosome abnormality, a reflex round of STR typing was required for 14.1% and 9.7% of the specimens analysed with Aneufast v2 and QST*Rplusv2, respectively (Table 1). When considering autosomal reflex testing alone this figure is reduced further to 5.9% for QST*Rplusv2. The finding of 5% is in line with the unpublished Saskatoon performance evaluation for the entire assessed chromosome complement (Table 2).

For the sex chromosomes, the difference in the amount of follow-up testing is greater between the assays. This is due to the inclusion in the initial PCR of the TAF9L paralogous marker in the QST*Rplusv2 assay. The TAF9L marker is an X-linked gene with paralogous sequence on chromosome 3. The chromosome-3-specific peak can therefore be used as the control for the reliable determination of X chromosome copy number. The incorporation of the TAF9L paralogous sequence in to the initial PCR design of the QST*Rplusv2 assay can therefore be considered advantageous for diagnostic use [1].

	QST*Rplusv2	Aneufast v2
No reflex	167 (90.3%)	159 (85.9%)
Reflex	18 (9.7%)	26 (14.1%)

*Table 1: Level of follow-up testing required per sample (% of those requiring follow-up testing for the QST*Rplusv2 assay versus the Aneufast v2 kit). These results were obtained from the assessment of 185 clinical samples by the The Molecular Laboratory within the University of Alberta Department of Medical Genetics, Canada[1]. Full details of methodology can be found in the referenced publication. The QST*Rplusv2 assay required less frequent reflex testing, which translates into shorter turnaround time and cost savings.*

	No reflex	Reflex
Chromosome 13	167 (90.3%)	2 (1.3%)
Chromosome 18	150 (99.3%)	1 (0.7%)
Chromosome 21	149 (98.7%)	2 (1.3%)
Chromosomes XY	148 (98.0%)	3 (2.0%)

Table 2: % of assessed cases requiring reflex testing reported per chromosome from the University Hospital of Saskatoon, Canada (unpublished data, personal communication). A total of 8 samples out of 151 analyses required reflex testing, representing 5.3% of cases. The majority of these cases represent reflex testing of the X and Y chromosomes, not the autosomes.

Alongside lower reflex testing requirements, the Alberta group found use of the QST*Rplusv2 kit saved time and money compared to the Aneufast v2 assay by way of a reduced need for troubleshooting. Overall, the group found that:

'**Although significantly improved compared with v1, the presence of dye blobs, particularly from the PET dye (in red), and differences in PCR efficiencies between S1 and S2 mixes[2] can still be problematic for some samples ran with Aneufast v2 (not shown). In our hands, separate S1 and S2 injections resulted in significant improvements in the electrophoresis profiles and consequently result interpretation. This, however, increases the cost to result**.'

CONCLUSION

The data presented here can help to provide guidance as to which commercial QF-PCR assay may best meets clinical laboratory testing needs and requirements. The performance of the QST*Rplusv2 QF-PCR assay was found to exceed that of the Aneufast™ v2 (MolgentixSL) kit with regard to the requirement for reflex testing and the associated reduction in costs and turnaround time. The group also report an easier workflow with reduced troubleshooting requirements, again representing cost and time savings. The reported rate of reflex testing is supported by unpublished data from the Saskatoon group, who similarly found the overall % of cases requiring reflex testing to be low. The superior performance of the assay is in part driven by the incorporation of the TAF9L paralogous sequence in to the initial PCR design. The overall design of the QSTRplusv2 assay can therefore be considered advantageous for diagnostic use.

1) https://pubmed.ncbi.nlm.nih.gov/22747196/
2) https://www.fsigeneticssup.com/article/S1875-1768(08)00225-4/fulltext

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