



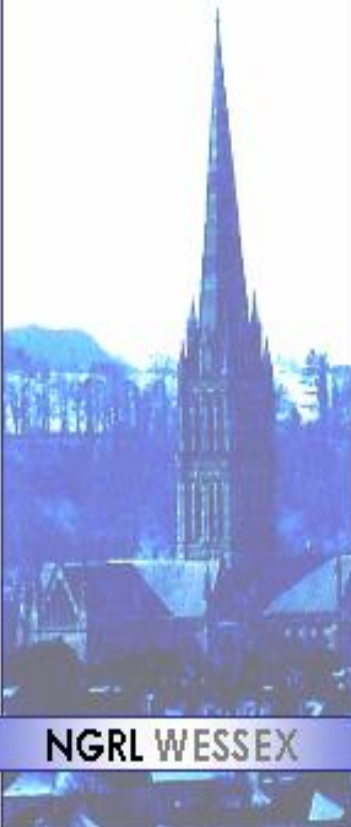
National Genetics Reference Laboratory
(Wessex)

NHS

**Technology
Assessment**


**ELUCIGENE QST*R™
(Tepnel Diagnostics)**

**for *in vitro* quantitative detection
of aneuploidy**



NGRL WESSEX

November 2006

Title	ELUCIGENE QST*R™ (Tepnel Diagnostics) for <i>in vitro</i> quantitative detection of aneuploidy
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Peer Review and Approval

This document has been reviewed by the study participants and an external expert. Tepnel Diagnostics have been given the opportunity to comment on the content of the report.

Conflicting Interest Statement

The authors declare that they have no conflicting financial interests

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List of Abbreviations

AF	Amniotic fluid
CE	Conformité Européene
CMGS	Clinical Molecular Genetics Society (UK)
CVS	Chorionic Villus
IVD	<i>In Vitro</i> Medical Devices Directive (98/79/EC)
MCC	Maternal cell contamination
NEQAS	National External Quality Assessment Service (UK)
NGRL	National Genetics Reference Laboratory
NHS	National Health Service (UK)
PSM	Primer binding site mutation / polymorphism
QF-PCR	Quantitative fluorescent polymerase chain reaction
rfu	Relative fluorescent unit
SMD	Submicroscopic duplication
SMM	Somatic microsatellite mutation
STR	Short tandem repeat

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ABSTRACT

NGRL (Wessex), in collaboration with six UK laboratories, has evaluated a QF-PCR based kit for the analysis of aneuploidy: ELUCIGENE QST*R™ (Tepnel Diagnostics). QST*R was developed in collaboration with Guy's and St Thomas' NHS Foundation Trust for the detection of trisomy 13, 18 & 21 with an additional kit (QST*R-XY) available for the detection of sex chromosome aneuploidy. The kit(s) is CE marked and therefore compliant with the *In Vitro* Medical Devices Directive (98/79/EC).

Initially, amniotic fluid (AF) DNA samples (n=243) and retrospectively collected aneuploid tissue DNA samples (n=88) were tested by NGRL (Wessex) in a blinded fashion. An additional 168 prenatal DNA samples were submitted by four UK laboratories who perform QF-PCR as a diagnostic service. These samples were tested by NGRL (Wessex) and included normal and aneuploid CVS and AF samples and problematic cases that labs had encountered using 'in house' primer sets e.g. those with maternal cell contamination, submicroscopic duplications, mosaicism, inconclusive allele ratios, primer binding site polymorphisms. All PCR products were analysed with an ABI 3100 and 3130 using Genotyper v3.7 and GeneMapper v3.7 respectively. Kits (50 reactions) were also sent to two other QF-PCR labs for them to trial in their laboratory using samples of their choice to compare the kits with existing diagnostic protocols and their comments are included in this report.

Over 95% of tests could have been reported without follow up studies being required. Use of the extra marker sets (QST*R-13,18 & 21) resolved single marker inconclusive allele ratios and provided extra informativity in all cases that required follow up. QST*R results were 100% consistent with the sample karyotype for the retrospectively collected DNA tissue samples and amniotic fluid samples from NGRL (Wessex). Follow up studies were not undertaken on the samples submitted from other laboratories but for the samples that did not require additional follow up studies the QST*R results were 100% consistent with the 'in house' QF-PCR results.

ELUCIGENE QST*R™ is technically easy to use, CE marked and therefore IVD compliant. It fulfils the typical requirements of a rapid prenatal test; the assay was accurate and no false positive results were obtained. The kits coped well with variable DNA quality, the results obtained were unambiguous and the failure rate was low. The manual supplied with the kit was very comprehensive and the instructions and advice were easy to follow. The inclusion of recommended electrophoresis conditions, machine settings, analysis macros and reporting sheets for the 3100 and 3130 Genetic Analysers minimises the amount of 'work-up' time required to implement the kit into diagnostic testing.

1. INTRODUCTION

Invasive prenatal diagnosis is offered routinely to pregnant women who have been identified as having an increased risk of foetal chromosome abnormalities. Pregnancies at high risk are identified by serum or ultrasound screening, advanced maternal age or because one parent is known to carry a chromosome abnormality. Invasive sampling takes place at either 10-12 weeks (chorionic villus sampling) or 15-20 weeks (amniocentesis) and diagnosis has traditionally been based on karyotype analysis which can detect both numerical and structural chromosome abnormalities. The most commonly detected abnormalities are trisomies for chromosome 21 (Down syndrome), chromosome 18 (Edwards syndrome), chromosome 13 (Patau syndrome) and sex chromosome aneuploidy (leading to syndromes such as Turner (monosomy X) and Klinefelter (XXY)). Karyotype analysis of chorionic villus (CVS) and amniotic fluid (AF) samples requires cell culture to obtain metaphase cells and skilled analysis of resulting banded chromosome preparations is also essential. Currently the UK average reporting times for full karyotype analysis are 13.5 days for AF (5.5% abnormality detection rate) with a range of 7.2 – 18.9 days and 14.8 days for CVS (16.9% abnormality detection rate) with a range of 7.9 – 23.6 days (UK NEQAS 2002/2003). In an effort to improve pregnancy management and alleviate maternal anxiety rapid aneuploidy detection techniques are now being implemented into routine prenatal diagnosis e.g. interphase FISH, quantitative fluorescent PCR (QF-PCR), multiplex ligation dependent amplification (MLPA). These tests are usually capable of delivering results within 1-3 days and are viewed as a prelude to, rather than a replacement of, full karyotype analysis.

Rapid prenatal aneuploidy tests need to fulfil certain criteria: the assay must be accurate and no false positive results should be obtained as this could result in the termination of a healthy pregnancy. The test should be robust enough to cope with variable sample quality, provide unambiguous results and have a low failure rate. Ambiguous results have the potential to increase maternal anxiety and can cause delays in reporting while additional investigations are carried out. The test should be adaptable to cope with high sample throughput and test costs should be low since rapid tests are often performed in addition to karyotyping. Ideally, the test should be able to detect maternal cell contamination (MCC), mosaicism and triploidy (Mann *et al.*, 2004).

QF-PCR analysis of short tandem repeats (STR) is being used successfully in many UK and European laboratories for the rapid diagnosis of prenatal aneuploidy (e.g. Verma *et al.*, 1998; Pertl *et al.*, 1999; Schmidt *et al.*, 2000; Cirigliano *et al.*, 2001; Levett *et al.*, 2001; Mann *et al.*, 2001). Chromosome specific polymorphic repeat sequences, which vary in length between individuals, are amplified using fluorescently labelled primers. The PCR amplicons are analysed using an automated genetic analyser capable of 2bp resolution and the representative amount of each allele is quantified by calculating the ratio of the peak height or area using appropriate software.

The ELUCIGENE QST*R kits have been developed in collaboration with Guy's and St Thomas' NHS Foundation Trust, London. The kits are CE marked and therefore compliant with the *In Vitro* Medical Devices Directive (98/79/EC). Five kits are available:

1. **QST*R**. Used for the routine detection of the three viable autosomal trisomies: trisomy 13, trisomy 18 and trisomy 21.
2. **QST*R-XY**. Used for the evaluation of sex chromosome status.
3. **QST*R-13**
4. **QST*R-18**
5. **QST*R-21**

QST*R-13, QST*R-18 and QST*R-21 are supplemental, and contain the chromosome specific markers from QST*R and additional chromosome specific markers. These kits can be used to provide additional information, to confirm an aneuploid result obtained using QST*R or for extended testing when a definitive result is not obtained using QST*R.

NGRL (Wessex) has evaluated QST*R, in collaboration with six UK laboratories. Initially, 243 amniotic fluid (AF) DNA samples and 88 retrospectively collected aneuploid tissue DNA samples were tested by NGRL (Wessex). An additional 168 prenatal DNA samples were then submitted by four network labs and tested by NGRL (Wessex). These samples included normal and aneuploid CVS and AF samples and problematic cases that labs had encountered e.g. those with maternal cell contamination, submicroscopic duplications, mosaicism, inconclusive allele ratios detected using 'in house' primer sets. All QST*R reactions were analysed with an ABI 3100 and 3130 using Genotyper v3.7 and GeneMapper v3.7 respectively. QST*R kits (50 reactions) were also sent to two other QF-

PCR laboratories for them to trial in their laboratory using samples of their choice to compare the kit with existing diagnostic protocols.

QST*R-XY was evaluated by analysing samples with sex chromosome aneuploidy selected from retrospectively collected tissue samples (n=21), NGRL (Wessex) amniotic fluid samples (n=8) and prenatal samples from the four UK labs (n=7).

2. MATERIALS AND METHODS

2.1 DNA Samples

2.1.1 Samples analysed with ELUCIGENE QST*R

2.1.1.1 Samples from NGRL (Wessex)

Retrospectively collected tissue DNA samples (**n=88**) from; normal controls (n=24), trisomy 18 (n=13), trisomy 13 (n=12), trisomy 21 (n=20) and sex chromosome aneuploidy (n=19) plus DNA samples from 1ml AF samples (**n=243**); normal (n=230), trisomy 13 (n=2), trisomy 21 (n=4), triploid (n=2), sex chromosome aneuploidy (n=2) and structural rearrangements (paternally inherited) (n=3) were tested using ELUCIGENE QST*R.

2.1.1.2 Samples from other UK laboratories

Four UK laboratories (Aberdeen, Birmingham, Bristol and Manchester) provided prenatal DNA samples (**n=168**) from AF (n=124), CVS (n=41), placental tissue (n=1) and POC (n=2) for analysis with ELUCIGENE QST*R™ at NGRL (Wessex). The samples had been tested previously by the referring lab using their 'in house' QF-PCR assays: QF-PCR and karyotype results were supplied for 100% and 86% of samples respectively. The samples were comprised of normal (n=70), trisomy 13 (n=7), trisomy 18 (n=11), trisomy 21 (n=43), triploidy (n=5, one case mosaic), sex chromosome aneuploidy (n=6, one case mosaic), as well as samples with submicroscopic duplications/ primer site polymorphisms (n=11) and cases with maternal cell contamination (n=15).

2.1.2 Samples analysed with ELUCIGENE QST*R-XY

DNA samples (**n=36**) from patients with sex chromosome aneuploidy; 45,X (n=13), 46,XX/46,XY (n=1), 45,X/46,XX (n=1), 46X, inv(X) (n=1), 47,XXX (n=2), 47,XXY (n=2), 48, XXYY (n=2), 48, XXXY (n=1), 49,XXXXY (n=2); triploid samples; 69,XXY (n=1), 69,XXX (n=1), mosaic triploid female (n=1), female mole (n=1) and normal controls (female, n=3; male n=4) were tested using ELUCIGENE QST*R-XY. DNA samples were selected from retrospectively collected tissue samples (n=21), NGRL (Wessex) amniotic fluid samples (n=8) and prenatal samples from the four UK labs (n=7).

2.2 DNA extraction

Retrospectively stored DNA samples had been extracted from a variety of tissues including; lymphoblastoid cell lines, skin, muscle, placenta and chorionic villi, cultured amniocytes, fibroblasts, urine, mouthbrush, blood and foetal tissue. 98% of prenatal samples from NGRL (Wessex) and the four UK laboratories had been extracted using InstaGene Matrix (BIORAD). DNA from 2% of prenatal samples was extracted using the EZ1 DNA tissue kit (QIAGEN) in conjunction with the BioRobot EZ1 Workstation (QIAGEN) or PureGene (placental tissue sample).

Reagents for extraction of DNA from amniotic fluid samples are not supplied with the kit although instructions are provided in the kit manual for DNA extraction with InstaGene Matrix (BIORAD). DNA samples were not quantified but provisional optimisation experiments confirmed that the use of 2.5µl of DNA prepared as outlined above produced robust results with the QST*R assays.

2.3 ELUCIGENE QST*R kit composition

QST*R and QST*R-XY are provided as single tube master mixes that contains PCR buffer, DNA polymerase, dNTPs and primer sets to amplify the STR markers listed in tables 1 and 2 respectively. The master mixes, sufficient for 50 reactions, can be dispensed into 10µl aliquots and stored at -20°C. Extra marker sets for chromosomes 13, 18 and 21 (QST*R-13, QST*R-18 and QST*R-21) are also available as single tube master mixes and amplify the STR markers listed in tables 3, 4 and 5 respectively. Examples of Genotyper traces generated using these kits are shown in appendix 1.

Marker	Chromosome Location	Observed Heterozygosity	Allele Size range (bp)	Marker Dye Colour
D13S252	13q12.2	0.85	260 – 330	red
D13S305	13q13.3	0.75	418 – 470	green
D13S628	13q31.1	0.69	425 – 472	yellow
D13S634	13q21.33	0.8	355 – 440	blue
D13S325	13q14.11	0.86	235 – 320	green
D18S386	18q22.1	0.88	320 – 407	green
D18S390	18q22.3	0.75	345 – 400	yellow
D18S391	18q11.31	0.75	196 – 230	green
D18S535	18q12.3	0.92	450 – 500	blue
D18S819	18q11.2	0.70	370 – 450	red
D18S978	18q12.3	0.67	180 – 230	yellow
D21S11	21q21.1	0.90	220 – 283	blue
D21S1437	21q21.1	0.84	307-343	blue
D21S1409	21q21.1	0.81	160 – 220	red
D21S1411	21q22.3	0.93	256 – 345	yellow
D21S1435	21q21.3	0.75	152 - 210	blue

Table 1: STR Marker information supplied in QST*R. STR marker, chromosome locations, amplified allele size ranges, fluorescent label and observed heterozygosity

Marker	Chromosome Location	Observed Heterozygosity	Allele Size range (bp)	Marker Dye Colour
DXS981	Xq13.1	0.86	225 – 260	blue
DXS1187	Xq26.2	0.72	122 – 170	green
HPRT	Xq26.2	0.78	265 – 300	green
DXS7423	Xq28	0.74	372 – 388	green
DXYS267	Xq21.3/Yp11.2	0.87	240 – 280	red
AMEL	Xp22.22/Yp11.2	-	104/110	yellow
DXS6807	Xp22.32	0.7	331 – 351	blue
DXS1283E	Xp22.31	0.89	292 – 340	yellow
SRY	Yp11.31	-	248	yellow
DYS448	Yq11.223	-	323 - 381	red

Table 2: STR Marker information supplied in QST*R-XY. STR marker, chromosome locations, amplified allele size ranges, fluorescent label and observed heterozygosity

Marker	Chromosome Location	Observed Heterozygosity	Allele Size range (bp)	Marker Dye Colour
D13S252	13q12.2	0.85	260 – 330	red
D13S305	13q13.3	0.75	418 – 470	green
D13S628	13q31.1	0.69	425 – 472	yellow
D13S634	13q21.33	0.8	355 – 440	blue
D13S325	13q14.11	0.86	235 – 320	green
D13S797	13q33.2	0.68	152-206/250	blue
D13S800	13q22.1	0.73	256 – 345	yellow
D13S762	13q31.3	0.8	260 – 350	blue

Table 3: STR Marker information supplied in QST*R-13. STR marker, chromosome locations, amplified allele size ranges, fluorescent label and observed heterozygosity

Marker	Chromosome Location	Observed Heterozygosity	Allele Size range (bp)	Marker Dye Colour
D18S386	18q22.1	0.88	320 – 407	green
D18S390	18q22.3	0.75	345 – 400	yellow
D18S391	18q11.31	0.75	196 – 230	green
D18S535	18q12.3	0.92	450 – 500	blue
D18S819	18q11.2	0.70	370 – 450	red
D18S978	18q12.3	0.67	180 – 230	yellow
D18S847	18q21.1	0.8	180 – 280	blue
D18S977	18q21.31	0.77	180 – 330	red
D18S1002	18q11.2	0.81	300 – 400	blue

Table 4: STR Marker information supplied in QST*R-18. STR marker, chromosome locations, amplified allele size ranges, fluorescent label and observed heterozygosity

Marker	Chromosome Location	Observed Heterozygosity	Allele Size range (bp)	Marker Dye Colour
D21S11	21q21.1	0.90	220 – 283	blue
D21S1437	21q21.1	0.84	307-343	blue
D21S1409	21q21.1	0.81	160 – 220	red
D21S1411	21q22.3	0.93	256 – 345	yellow
D21S1435	21q21.3	0.75	152 - 210	blue
D21S1442	21q21.3	0.96	290 - 360	green
D21S1446	21q22.3	0.71	180 - 250	green

Table 5: STR Marker information supplied in QST*R-21. STR marker, chromosome locations, amplified allele size ranges, fluorescent label and observed heterozygosity

2.4 Multiplex PCR amplification

2.5µl DNA (1.25 – 10 ng) was added to 10µl QST*R (-XY, -13, -18, 21) reaction mix (12.5µl final reaction volume) and amplified using the PCR conditions specified in the ELUCIGENE QST*R™ Instructions for Use:

95°C	15 min	} 26 cycles
95°C	30 sec	
59°C	1.5 min	
72°C	1.5 min	
72°C	30 min	

2.5 Electrophoresis of amplified products

Each amplicon was analysed using an ABI 3100 and ABI 3130 Genetic Analyzer (Applied Biosystems). For 16 analyses 6.85µl of GS500 LIZ size standard (Applied Biosystems) was added to 250µl Hi-Di Formamide (Applied Biosystems) and 15µl of the mix was dispensed into each well of a 96 well plate. 3µl of the PCR product was added to the size standard and the plate sealed. The PCR products were then denatured at 94°C for 3 minutes and cooled at 4°C for 30 seconds. Samples were then loaded onto the genetic analysers using parameters and procedures which are comprehensively presented in the ELUCIGENE QST*R Instructions for Use (www.elucigene.com).

2.6 Data analysis and interpretation

The data collected were analysed with Genotyper 3.7 and GeneMapper 3.7 (Applied Biosystems) using macros available from ELUCIGENE and all profiles were examined manually. A maximum of two peaks are labeled automatically for each STR marker. If three alleles are present, the peak must be labeled manually. Tabulated data from Genotyper 3.7 and GeneMapper 3.7 were imported into QST*R report templates (specially configured Excel spreadsheets) which assist with data analysis (figure 1). Spreadsheets and macros can be downloaded from the ELUCIGENE website www.elucigene.com.

Data were analysed and interpreted in accordance with the Clinical Molecular Genetics Society (CMGS) best practice guidelines (<http://www.cmgs.org/BPG/Guidelines/2004/QFPCR.htm>) and following interpretation guidance supplied in the QST*R Instructions for Use. Allele dosage ratios (assessed using peak area) between 0.8 – 1.4 were defined as normal (a ratio of 1.5 was considered acceptable if the alleles were separated by more than 24bp), ratios of >1.8 or <0.65 were considered to be indicative of trisomy and the presence of three alleles of equal peak height was also considered to be indicative of trisomy (figure 2). Allele ratios that fell between the normal and abnormal ranges were termed inconclusive. An allele ratio was termed inconsistent if the ratio suggested the presence of three alleles when other informative markers for the same chromosome demonstrated normal di-allelic ratios. The presence of a single peak was defined as uninformative and a minimum of two informative markers were considered necessary for confident interpretation. Markers with allele peaks below 50 relative fluorescent units (rfu) or above 6000 rfu were excluded from the analysis.

For this evaluation, when an inconclusive allele ratio was obtained for a single marker on a chromosome, where 2 or more markers on the same chromosome (i.e. all informative markers) showed normal allele ratios, the traces were carefully examined for evidence of mosaicism. If there was no evidence of mosaicism the sample was reported as normal.

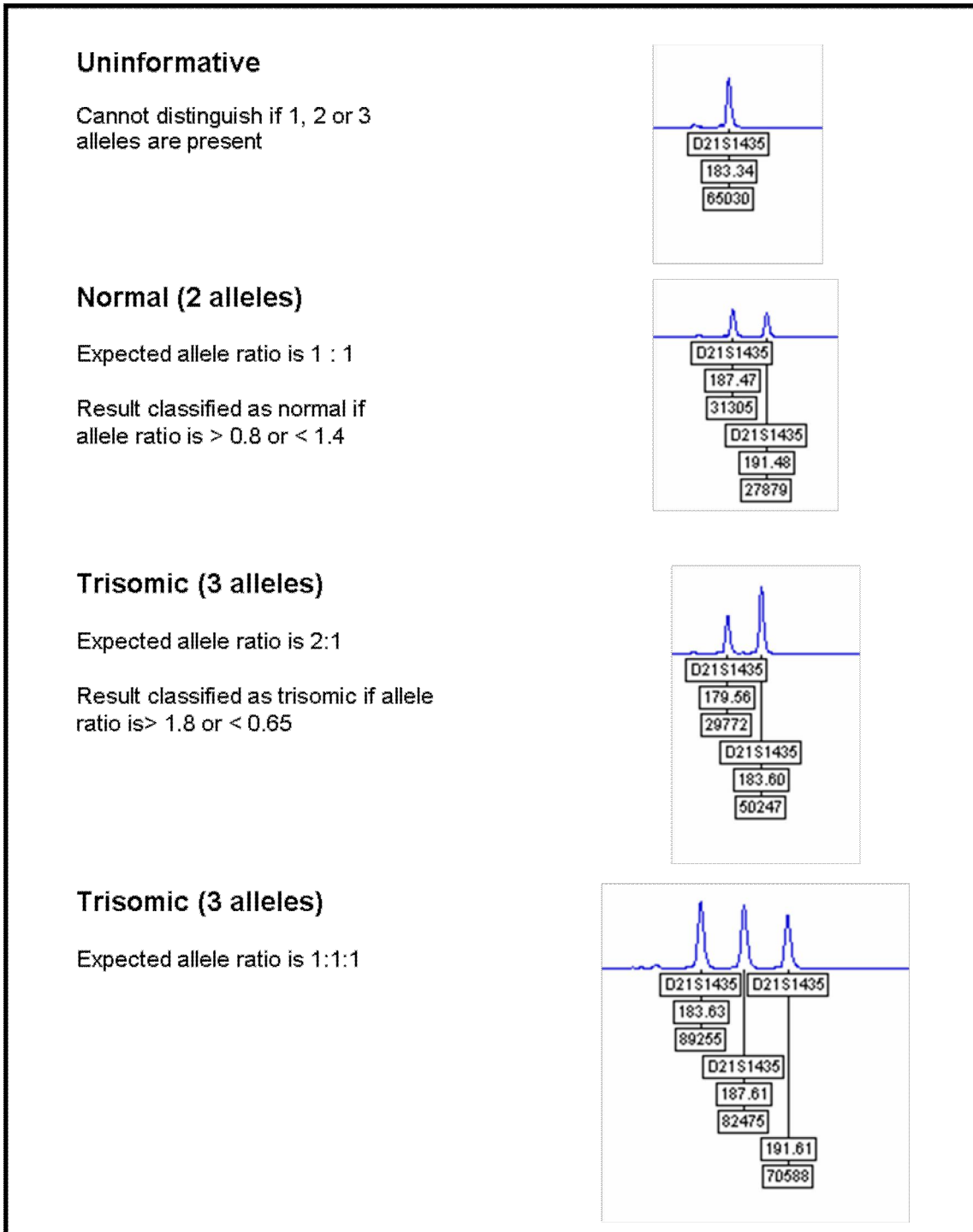


Figure 1: Interpretation of QST*R Data. Allele ratios can be uninformative, normal (2 alleles with a 1:1 ratio), trisomic (3 alleles) or trisomic (3 alleles with a 2:1 ratio)

3. RESULTS

3.1 NGRL (Wessex) samples analysed with QST*R

3.1.1 Marker Heterozygosity

The heterozygosity of the autosomal markers was determined from the analysis of 243 amniotic fluid samples referred to the Wessex Regional Genetics Laboratory (table 6).

Marker	Chromosome Location	Allele Size range (bp)	Reported Heterozygosity	NGRL (W) observed heterozygosity
D13S252	13q12.2	260 – 330	0.85	0.72
D13S305	13q13.3	418 – 470	0.75	0.84
D13S628	13q31.1	425 – 472	0.69	0.8
D13S634	13q21.33	355 – 440	0.80	0.91
D13S325	13q14.11	235 – 320	0.86	0.81
D18S386	18q22.1	320 – 407	0.88	0.95
D18S390	18q22.3	345 – 400	0.75	0.69
D18S391	18q11.31	196 – 230	0.75	0.63
D18S535	18q12.3	450 – 500	0.92	0.74
D18S819	18q11.2	370 – 450	0.70	0.78
D18S978	18q12.3	180 – 230	0.67	0.74
D21S11	21q21.1	220 – 283	0.90	0.81
D21S1437	21q21.1	283 – 350	0.84	0.71
D21S1409	21q21.1	160 – 220	0.81	0.74
D21S1411	21q22.3	256 – 345	0.93	0.86
D21S1435	21q21.3	152 - 210	0.75	0.79

Table 6: Heterozygosity for QST*R markers

3.1.2 Inconclusive Allele Ratios

Table 7 shows the frequency of inconclusive allele ratios (*i.e.* the allele ratio falls between 0.65 and 0.8 or between 1.4 and 1.8) detected for each autosomal marker when analysed on the ABI 3100 and 3130 Genetic Analysers. Five retrospectively collected samples showed inconclusive allele ratios for single markers when analysis was undertaken using the ABI 3100 and 3130. 30 amniotic fluid samples (12.3%) showed inconclusive allele ratios when analysed on an ABI 3100. Six of these had more than one marker showing an inconclusive allele ratio. 32 amniotic fluid samples (13.1%) showed inconclusive allele ratios when analysed on an ABI 3130. Ten of these had more than one marker showing an inconclusive allele ratio. 22 amniotic fluid samples were identified as having the same inconclusive allele ratios for the same markers when analysed on both the 3100 and 3130. 18 amniotic fluid samples showed discrepancies between analysis on the 3130 and 3100 with inconclusive allele ratios being observed on one platform only; ABI 3100 (n=7) and ABI 3130 (n=11).

The marker which produced inconclusive ratios in the highest number of samples (4%) was D18S386. In all cases the alleles were separated by more than 20bp (range 20-48bp) and the ratios varied from 1.46 – 1.77. For this marker it would be useful to have a statistical analysis which involved measurement of standard deviation based on allele separation (combined with peak heights and areas) to define acceptable ratios for widely spaced alleles.

Marker	Chromosome Location	Allele Size range (bp)	Retrospectively collected samples (n=88)		Prospectively collected amniotic fluid samples (n=243)	
			3100	3130	3100	3130
D13S252	13q12.2	260 – 330	-	-	-	0.4% (1)
D13S305	13q13.3	418 – 470	-	-	1.6% (4)	2.0% (5)
D13S628	13q31.1	425 – 472	-	-	0.8% (2)	0.4% (1)
D13S634	13q21.33	355 – 440	-	1.1% (1)	1.2% (3)	1.6% (4)
D13S325	13q14.11	235 – 320	-	-	0.4% (1)	-
D18S386	18q22.1	320 – 407	2.3% (2)	1.1% (1)	4.0% (10)	4.0% (10)
D18S390	18q22.3	345 – 400	-	-	0.4% (1)	-
D18S391	18q11.31	196 – 230	-	-	1.2% (3)	0.8% (2)
D18S535	18q12.3	450 – 500	-	-	1.6% (4)	2.8% (7)
D18S819	18q11.2	370 – 450	-	1.1% (1)	0.4% (1)	0.8% (2)
D18S978	18q12.3	180 – 230	-	-	0.4% (1)	-
D21S11	21q21.1	220 – 283	-	-	-	-
D21S1437	21q21.1	283 – 350	-	-	0.4% (1)	0.8% (2)
D21S1409	21q21.1	160 – 220	1.1% (1)	-	1.6% (4)	2.5% (6)
D21S1411	21q22.3	256 – 345	1.1% (1)	-	0.8% (2)	1.6% (4)
D21S1435	21q21.3	152 - 210	-	-	0.4% (1)	-

Table 7: Percentage of inconclusive allele ratios obtained for QST*R markers for retrospectively and prospectively collected samples. The number of samples demonstrating inconclusive allele ratios are given in brackets.

Analysis of marker D21S1409 on the ABI 3130 was sometimes problematic due to the presence of a dye blob (at c.190bp) within the expected allele size range. The dye blob was present at varying heights in most samples and caused skewing of allele ratios in 2% of cases analysed on the ABI 3130 (e.g. figure 2).

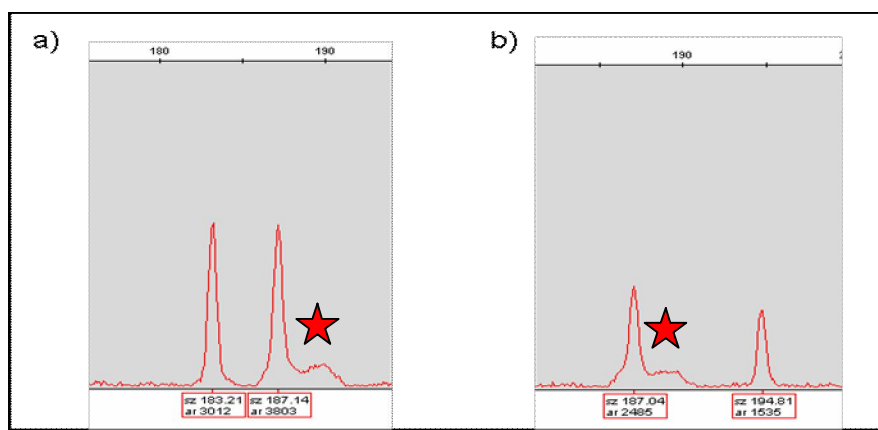


Figure 2: Marker D21S1409. A dye blob at 190bp (indicated by red stars) caused problems for analysis as the area of the blob was often incorporated into the area of the allele specific peak resulting in inconclusive allele ratios. a) The peak height ratio for this sample was 1.0 but the presence of the dye blob has caused skewing of the peak area allele ratio to 0.79. b) The peak height ratio for this sample was 1.22 but the presence of the dye blob has skewing of the peak area allele ratio to 1.61. This was only observed when using the 3130 Genetic Analyser (Applied Biosystems).

3.1.3 QST*R results for retrospectively collected tissue samples (n=88)

Results for this section are summarised in table 8

3.1.3.1 Retrospectively collected tissue samples (n=88) analysed using ABI 3100

The QST*R results were consistent with the sample karyotype in 100% of cases (n=88). 84 samples (95%) could be confidently interpreted without any further testing being required. Four samples (5%) required additional tests to be carried out for the following reasons:

- **Informativity (n=1)**

1 sample had only one marker informative for chromosome 21. This sample was retested using QST*R-21 and the sample had two informative markers which had normal allele ratios.

- **Inconclusive allele ratios (n=3)**

2 samples had allele ratios that were consistent with trisomy 18 but marker D18S386 showed an inconclusive allele ratio in both cases (0.68 & 0.77). When these samples were retested with QST*R-18 the allele ratios for D18S386 and additional markers were consistent with trisomy 18.

1 sample had allele ratios that were consistent with trisomy 21 but marker D21S1411 showed an inconclusive allele ratio (0.71). When this sample was retested with QST*R-21 the allele ratio for D21S1411 was consistent with trisomy 21 (0.61)

3.1.3.2 Retrospectively collected tissue samples (n=88) analysed using ABI 3130

The QST*R results were concordant with the karyotype for 100% of samples (n=88). 83 samples (94.3%) could be confidently interpreted without any further testing being required. Five samples (5.7%) required additional tests to be carried out for the following reasons:

- **Informativity (n=1)**

1 sample had only one marker informative for chromosome 21. This sample was retested using QST*R-21 and the sample had two informative markers which had normal allele ratios.

- **Inconclusive allele ratios (n=4)**

3 samples had allele ratios that were consistent with trisomy 18 where marker D18S386 showed an inconclusive allele ratio in two cases (0.70 & 0.73) and marker D18S189 in another (0.67). When these samples were retested with QST*R-18 the allele ratios for all informative markers were consistent with trisomy 18.

1 sample had allele ratios that were consistent with trisomy 21 but marker D21S1411 showed an inconclusive allele ratio (0.66). When this sample was retested with QST*R-21 the allele ratio for D21S1411 was consistent with trisomy 21 (0.61).

Results	3100 (n=88)	3130 (n=88)
QST*R Result consistent with karyotype:	88 (100%)	88 (100%)
i) no additional tests required	84 (95.5%)	83 (94.3%)
ii) extra markers tested for informativity	1 (1.1%)	1 (1.1%)
iii) single marker repeats	3 (3.4%)	4 (4.5%)

Table 8: Summary of QST*R results obtained from retrospectively collected DNA samples (n=88) analysed using 3100 and 3130 Genetic Analysers (Applied Biosystems).

3.1.4 QST*R results for NGRL (Wessex) amniotic fluid samples (n=243)

3.1.4.1 Prospectively collected amniotic fluid samples (n=243) analysed using ABI 3100

The QST*R results were concordant with the karyotype for 100% of samples (n=242) with one sample failing. 234 samples (96.3%) could be confidently interpreted without any further testing being required. 9 samples (3.7%) required additional tests to be carried out for the following reasons:

- **Informativity (n=4)**

3 samples had only one marker informative for chromosome 21. These samples were retested using QST*R-21 and the samples had two informative markers which had normal allele ratios.

1 sample had only one marker informative for chromosome 13. This sample was retested using QST*R-13 and the sample had two informative markers which had normal allele ratios.

- **Inconsistent allele ratios (n=1)**

1 sample was triallelic for marker D21S1411 with other markers for this chromosome having normal allele ratios. In addition, marker D18S390 was uninformative. In rare cases the allele size ranges for D21S1411 and D18S390 may overlap. The sample was retested using QST*R-18 that confirmed that the extra allele, originally classified as D21S1411, was a D18S390 allele. The resulting allele ratio for D18S390 was normal.

- **Inconclusive allele ratios (n=4)**

1 sample had inconclusive allele ratios for D18S386 (1.61, alleles 44bp apart) and D18S535 (1.61, alleles 12bp apart). The sample was repeated using QST*R-18 and all informative markers (n=5) had normal allele ratios (D18S386, 1.46; D18S535 0.97).

1 sample had inconclusive allele ratios for D18S386 (1.75, alleles 48bp apart), D18S535 (1.48, alleles 8bp apart) and D18S819 (1.79, alleles 12bp apart). The samples was repeated using QST*R-18 and all informative markers (n=9) had normal allele ratios (D18S386, 1.5).

1 sample had inconclusive allele ratios for D18S391 (1.54, alleles 4bp apart) and markers D18S535 and D18S819 failed as they had peak heights <50 rfu. The sample was repeated using QST*R-18 but the reaction failed.

1 sample had one informative marker (D21S1411, 0.99) and an inconclusive ratio for D21S1435 (1.42, alleles 4bp apart) with all other chromosome 21 markers being uninformative. The sample was repeated using QST*R-21 and all informative markers (n=4) had normal allele ratios (D21S1435, 1.10).

3.1.4.2 Prospectively collected amniotic fluid samples (n=243) analysed using ABI 3130

The QST*R results were concordant with the karyotype for 100% of samples (n=243). 235 samples (96.7%) could be confidently interpreted without any further testing being required. 8 samples (3.3%) required additional tests to be carried out for the following reasons:

- **Informativity (n=4)**

3 samples had only one marker informative for chromosome 21. These samples were retested using QST*R-21 and the samples had two informative markers which had normal allele ratios.

1 sample had only one marker informative for chromosome 13. This sample was retested using QST*R-13 and the sample had two informative markers which had normal allele ratios.

- **Inconsistent allele ratios (n=1)**

1 sample was triallelic for marker D21S1411 with other markers for this chromosome having normal allele ratios. In addition, marker D18S390 was uninformative. In the Instructions for Use it states that: " In rare cases, it has been observed that the allele size ranges for D21S1411 and D18S390 can overlap. This results in the shorter length allele of D18S390 being labelled as a D21S1411 allele. Due

to the dynamic nature of STR alleles, this may occur rarely with other markers leading to mislabelling of alleles. If this is suspected, analysis with the single chromosome kits may resolve this.” The sample was retested using QST*R-18 which confirmed that the extra allele, originally classified as D21S1411, was a D18S390 allele. The resulting allele ratio for D18S390 was normal.

- **Inconclusive allele ratios (n=3)**

1 sample had inconclusive allele ratios for D18S386 (1.65, alleles 44bp apart) and D18S535 (1.6, alleles 12bp apart). The sample was repeated with QST*R-18 and all informative markers (n=5) had normal allele ratios (D18S386, 1.44; D18S535, 1.01).

1 sample had inconclusive allele ratios for D18S386 (1.78, alleles 48bp apart), D18S535 (1.46, alleles 8bp apart) and D18S819 (1.73, alleles 12bp apart). The samples was repeated using QST*R-18 and all informative markers (n=9) had normal allele ratios except D18S386 which retained an inconclusive allele ratio of 1.58.

1 sample had only one informative marker (D21S1411, 1.08) and an abnormal ratio for D21S1409 (0.44, alleles 12bp apart) with all other markers on chromosome 21 being uninformative. The sample was repeated using QST*R-21 and all informative markers (n=2) had normal allele ratios (D21S1409, 1.10).

Results	Amniotic fluid samples (n=243)	
	3100	3130
QST*R Result consistent with karyotype:	243 (100%)	243 (100%)
i) no additional tests required	234 (96.3%)	235 (97.1%)
ii) extra markers tested for informativity	4 (1.6%)	4 (1.6%)
iii) single marker repeats	5 (2%)	4 (1.6%)

Table 9: Summary of QST*R results obtained from amniotic fluid samples (n=243) analysed using 3100 and 3130 Genetic Analysers (Applied Biosystems).

3.1.5 QST*R results for prenatal samples sent from UK labs (n=168)

Prenatal samples were submitted from 4 UK laboratories who offer QF-PCR detection of aneuploidy as a diagnostic service. The samples had been tested previously by the referring lab using their ‘in house’ QF-PCR assays. Results from ‘in house’ QF-PCR assays and karyotypes were supplied for 100% and 86% of samples respectively. The samples included normal and aneuploid CVS and AF samples and problematic cases that labs had encountered e.g. those with maternal cell contamination, submicroscopic duplications, mosaicism and inconclusive allele ratios detected using ‘in house’ primer sets. Therefore, this set of samples is not representative of the majority of samples analysed on a daily basis. Analysis of the DNA samples was undertaken by NGRL (Wessex). Apparent differences in DNA quality were observed between labs which may be due to many factors including variation in transportation conditions and difference in the way the samples were processed before sending e.g. received in Instagene matrix or as supernatant only. To account for these differences the data will be presented for each laboratory individually. Follow up studies were not undertaken on these samples to resolve single marker inconclusive allele ratios etc. Results from this section are summarised in table 10.

3.1.5.1 Samples from lab 1 (AF n=36; CVS n=14)

In house QF-PCR results for the DNA supplied from this lab were: normal (n = 31), trisomy 13 (n=2), trisomy 18 (n=2), trisomy 21 (n = 6, one case with only one informative marker for 18), monosomy X (n=1), triploid (n=2), MCC (n=4), SMM for D13S631 (n=1) and submicroscopic duplication for D13S634 (n=1).

- Normal samples (n=31)

Of the samples with normal 'in house' QF-PCR results, 26 (84%) had the expected normal allele ratios when tested using QST*R. Four samples amplified weakly and could not be interpreted confidently, one sample showed a normal allele ratios when using the 3100 but five markers had inconclusive allele ratios when the sample was analysed using the 3130 (D13S634, D18S386, D18S390, D21S1409, D21S1435).

- Aneuploid and triploid samples (n=13)

The QST*R results were concordant with the 'in house' QF-PCR results in 11 cases (85%). For the trisomy 21 case that showed only one informative marker for chromosome 18 when using the 'in house' assay: 3 QST*R markers for chromosome 18 were informative. Two trisomy 21 samples would have required follow up studies; one sample had inconclusive allele ratios for D13S634, D13S325 and D18S535 with all chromosome 21 markers having allele ratios indicative of trisomy 21 and the other sample had a normal allele ratio for marker D21S1411 (3100 and 3130) with all other markers having allele ratios indicative of trisomy 21.

- MCC, SMM and submicroscopic duplications (n=6)

The QST*R results were concordant with the 'in house' QF-PCR results in all cases. Marker D13S631 is not included in QST*R but the submicroscopic duplication for D13S634 was observed (see appendix 2). An example of a Genotyper trace showing a maternal cell contamination case from lab 1 is also shown in appendix 2.

3.1.5.2 Samples from lab 2 (AF n=28; CVS n=15, placental tissue n=1)

In house QF-PCR results for the DNA supplied from this lab were: normal (n = 20; 1 sample SMM for D13S628, 1 sample unstable for D18S386), trisomy 13 (n=1), trisomy 18 (n=5), trisomy 21 (n = 8), monosomy X (n=3), triploid (n=1), MCC (n=5) and submicroscopic duplication for D13S742 (n=1).

- Normal samples (n=21)

The QST*R results were concordant with the 'in house' QF-PCR results in all cases. The case identified as being D13S628 unstable by the 'in house' QF-PCR assay was uninformative for D13S628 using QST*R. A somatic microsatellite mutation for D18S386 was observed using QST*R in the sample reported as D18S386 unstable by lab 2 (see appendix 2).

- Aneuploid and triploid samples (n=17)

The QST*R results were concordant with the 'in house' QF-PCR results in 16 (94%) cases. One case may have required follow up as only two markers on chromosome 13 were informative with one having an inconclusive allele ratio.

- MCC and submicroscopic duplications (n=6)

The QST*R results were concordant with the 'in house' QF-PCR results in all cases. Marker D13S742 is not included in QST*R and so the sample with the submicroscopic duplication was interpreted as normal. An example of a maternal cell contamination case from lab 2 is shown in appendix 2.

3.1.5.3 Samples from lab 3 (AF n=14, CVS n=8, POC n=2)

In house QF-PCR results for the DNA supplied from this lab were: normal (n = 13, 1 case required extra markers for 13, 4 cases required extra markers for 18, 4 cases required extra markers for 21), trisomy 13 (n=1), trisomy 18 (n=2), trisomy 21 (n = 3, one required extra 21 markers), mosaic triploid (n=1), MCC (n=1), female mole (n=1), somatic microsatellite mutation at D21S11 (n=1), possible primer binding site polymorphism for D18S391 (n=1).

- Normal samples (n=13)

One sample failed to amplify. For those which amplified successfully, the QST*R results were concordant with the 'in house' QF-PCR results in 10 cases (83%). In all cases where extra markers were required to increase the number of informative loci, the sample had at least two informative

markers for the chromosome when tested using QST[®]R. Two cases would have required follow up studies; one sample showed an unusual profile with some markers amplifying more strongly than others and marker D21S1409 failed to amplify. The Genotyper trace for this sample is shown in appendix 2 (BRL3). Similar traces have been observed by another lab and the 'dropout' pattern appears to be due to tube specific set up problems such as inaccurate pipetting or insufficient mixing rather than DNA or kit quality. They have shown that repeating the PCR resolves this problem although we did not repeat the PCR in this case. The other case showed inconclusive allele ratios for D13S628 and D13S634. This sample had amplified weakly and would need to be repeated.

- Aneuploid and triploid samples (n=7)

The QST[®]R results were concordant with the 'in house' QF-PCR results in all cases. The Genotyper trace for the mosaic triploid case is shown in appendix 2 (BRL15).

- Other (n=4)

The QST[®]R results were concordant with the 'in house' QF-PCR results in all cases. The Genotyper traces for the female mole, somatic microsatellite mutation at D21S11 and possible primer binding site polymorphism for D18S391 (n=1) are shown in appendix 2 (BRL24, 21 and 19).

3.1.5.4 Samples from lab 4 (AF n=46, CVS n=4)

'In house' QF-PCR results for the DNA supplied from this lab were: normal (n = 11), trisomy 13 (n=3), trisomy 18 (n=3), trisomy 21 (n = 25), triploid (n=1), failed (MCC) (n=5), primer site polymorphism D21S1411 (n=1) and submicroscopic duplication D13S742 (n=1). The quality and/or concentration of DNA from this lab differed to other lab samples and a high number of samples failed to amplify or amplified with weaker profiles than DNA samples from other centres (24% failure rate, 40% had at least 4 markers with peaks <100rfu).

- Normal samples (n=11)

Two cases failed to amplify. For the cases which amplified successfully, the QST[®]R results were concordant with the 'in house' QF-PCR results in 7 cases (78%). Two cases would have required follow up. One sample had two informative markers for chromosome 18 but one of these (D18S819) had an inconclusive allele ratio. The other sample had low input DNA and the higher molecular weight markers had signals <100rfu also, the D13S252 allele ratio was inconclusive and the D18S386 allele ratio was abnormal (2.0) on both the 3130 and 3100.

- Aneuploid and triploid samples (n=32)

Nine cases failed to amplify sufficiently to be interpreted (28%). For the cases which amplified successfully, the QST[®]R results were concordant with the 'in house' QF-PCR results in 16 cases (70%). Seven cases would have required follow up studies:

One case with trisomy 18 amplified weakly and markers D13S305 and D13S628 had peak heights <50rfu meaning that only one informative marker with a normal allele ratio was found on chromosome 13. Three markers for chromosome 18 were informative and 2 showed allele ratios consistent with trisomy 18 with the other marker (D18S386) having an inconclusive allele ratio (0.67, 3100; 0.7 3130).

Two cases with trisomy 21 had inconclusive and inconsistent allele ratios at markers on chromosome 13 and 18 and appeared to have maternal cell contamination.

Two cases of trisomy 21 had inconclusive allele ratios for marker D21S1411 (case 1: 0.78 (3100), 0.74 (3130); case 2: 0.67 (3100), 0.68 (3130) with all other markers on 21 being consistent with trisomy 21.

One case with trisomy 21 had and an inconsistent allele ratio for marker D13S305 (1.83, 3100; 1.9, 3130).

One case with trisomy 21 had an inconsistent allele ratio for marker D18S819 (0.56, 3100; failed 3130).

- MCC, PSM and submicroscopic duplications (n=7)

The sample with an 'in house' QF-PCR result indicating a primer site polymorphism at D21S1411 failed to amplify. For samples that amplified, the QST*R results were concordant with the 'in house' QF-PCR results in all cases. Marker D13S742 is not included in QST*R and so the sample with the submicroscopic duplication was interpreted as normal.

Lab	Samples that amplified	QST*R results consistent with 'in house' QF-PCR result after initial testing	Follow up required
1	Combined (n=46)	43 (93.5%)	3 (6.5%)
	Normal (n=27)	26 (96.3%)	1 (3.7%)
	Aneuploid or triploid (n=13)	11 (84.6%)	2 (15.4%)
	PSP/SMM/SMD and other (n=6)	6 (100%)	-
2	Combined (n=44)	43 (97.7%)	1 (2.7%)
	Normal (n=21)	21 (100%)	-
	Aneuploid or triploid (n=17)	16 (94.1%)	1
	PSP/SMM/SMD and other (n=6)	6 (100%)	-
3	Combined (n=23)	21 (91.3%)	2 (8.7%)
	Normal (n=12)	10	2
	Aneuploid or triploid (n=7)	7	-
	PSP/SMM/SMD and other (n=4)	4	-
4	Combined (n=38)	29 (76.3%)	9 (23.7%)
	Normal (n=9)	7	2
	Aneuploid or triploid (n=23)	16	7
	PSP/SMM/SMD and other (n=6)	6	-
Overall (excluding lab 4)	All samples tested (n=151)	136 (90%)	15 (10%)
	(n=113)	107 (95%)	6 (5%)

Table 10: Summary of the QST*R results obtained from 'unusual' prenatal samples referred from four UK laboratories that amplified using QST*R. Failure rates were 8%, 0%, 4% and 24% for labs 1-4 respectively. QST*R data have been compared with 'in house' PCR results.

3.1.6 QST*R-XY results analysed using 3100 and 3130 (n=36)

Some centres consider that it is only appropriate to test for sex chromosome aneuploidy in a subset of referrals. Such decisions are usually made at a local level after consultation with clinical colleagues. QST*R-XY can be used to evaluate sex chromosome aneuploidy either routinely alongside QST*R or in a subset of referrals as requested by clinicians.

CMGS best practice guidelines state that:

“It is important to be aware that the QF-PCR sex chromosome assay (Donaghue *et al.*, 2003) is a highly stringent screen for monosomy X but not a diagnostic test. A result consistent with monosomy X, where all polymorphic markers have only a single allele peak and no Y sequences are present, may represent a normal female homozygous for all markers tested. Therefore it is **recommended** that the result is either confirmed using another technique, or that it is reported as being consistent with monosomy X with the caveat that there remains a possibility that a normal female could give the same genotype.”

Information provided in the Instructions for Use for QST*R-XY states that “a result exhibiting no amplification for Y specific markers and homozygous for all other markers is not necessarily diagnostic of Turner syndrome. Approximately 1 in 171,000 females will be homozygous for all 7 X specific polymorphic markers. This gives a Bayesian probability of approximately 1 in 1170 that a profile homozygous for all X specific markers represents a true monosomy X genotype rather than a normal homozygous female.”

We tested retrospectively collected tissue DNA samples and prenatal DNA samples (n=36) using QST*R-XY. The data obtained from analysis of the amplicons with the 3100 and 3130 Genetic Analysers were 100% concordant. QST*R-XY data were consistent with the sample karyotype in all cases (or highly indicative in the case with a karyotype of 49, XXXXY). One case, which had a karyotype consistent with Turner syndrome (45,X ; 30 cells analysed) showed mosaicism for Y chromosome derived material at a level of approximately 10% in uncultured cells tested with QST*R-XY (see Genotyper trace; appendix 3). Similar findings have been reported in Turner syndrome cases which show absence of a Y chromosome (or marker) at the cytogenetic level (e.g. Mazzanti *et al.*, 2005). Reports of Y chromosome sequences in patients with Turner syndrome have been reported to range from 0% (Larsen *et al.*, 1995) to 61% (Coto *et al.*, 1995) with studies showing variation in the sensitivities of techniques, cell populations analysed, differences in Y sequences detected and possible selection bias. Follow up studies could not be undertaken on this case because all samples were anonymised prior to testing.

Examples of Genotyper traces for a variety of sex chromosome aneuploidy samples are shown in appendix 3.

3.1.7 Samples analysed with QST*R-13, QST*R-18 and QST*R-21

The extra markers supplied with QST*R performed well. Samples were tested with QST*R-21 (n=14, 3100; n=17, 3130), QST*R-18 (n=19, 3100; n=21, 3130) and QST*R-13 (n=11, 3100; n=7, 3130) using either the 3100 or 3130. Samples were selected because they had shown inconclusive or inconsistent allele ratios when tested with QST*R. In all cases re-testing of the samples with the extra markers resolved the allele ratio discrepancies. However, in some cases other markers within the multiplex then became skewed. It is therefore important to define whether only the single marker that is being re-tested is analysed or whether all data should be analysed. Occasionally the additional markers (those not included in QST*R) showed inconclusive allele ratios with the other markers being normal which could complicate follow up studies. Defining analysis parameters is also important when the annealing temperature of the reaction is dropped to resolve possible primer binding site mutations as this often results in skewing of markers that previously gave normal allele ratios. When the markers are being used to provide extra informativity or confirm a trisomy result then all markers are analysed. Examples of Genotyper traces for the extra marker kits are shown in appendix 4.

3.2 General Comments on ELUCIGENE QST*R

A comprehensive manual is supplied with ELUCIGENE QST*R™ which covers all aspects of the use of the kit including advice on DNA extraction, PCR set-up, electrophoresis conditions, data analysis and data interpretation. The manual is easy to follow and the inclusion of recommended electrophoresis conditions and analysis macros for the 3100 and 3130 Genetic Analysers minimises the amount of 'work-up' time require to implement the kit into diagnostic testing. The Excel spreadsheets which are provided to help with analysis are very useful (appendix 1).

The PCR reactions were extremely easy to set up and the Genotyper/GeneMapper traces that were produced were of good quality with low or absent background and there were no non-specific peaks (except the dye blob for D21S1409 using the 3130). The primer concentrations appear to be well balanced so that all markers amplify with similar intensity for the majority of samples.

We noticed that the peak morphology for the 3100 and 3130 analysis differed and that more samples showed inconclusive allele ratios for single markers when analysed using the 3130 compared to the 3100. When markers that showed normal allele ratios on the 3100 but inconclusive allele ratios on the 3130 were looked at in more detail we noticed that if these were analysed using peak height the allele ratios were normal. Although this had little impact on the overall data quality it would be useful to investigate this further as it may be possible to modify how the peak areas are assessed when analysing data with GeneMapper v3.7.

3.3 Comments and data from other laboratories who tested QST*R

3.3.1 Liverpool Molecular Genetics

Overall, we were very impressed with the QST*R kit for detection of trisomy 13, 18 & 21. We found the protocol to be very straightforward and easy to follow. The assay has been designed with a minimum hands-on set up time, which is obviously very important for a diagnostic service such as this with a very tight turnaround time. The format of the QST*R kit is very similar to our existing in-house assay and so if we were to use it on a routine basis, it would fit in very well with existing laboratory procedures.

The supporting materials were of high quality. The 'Instructions for Use' were well presented and gave all the necessary information in an easily accessible format. In fact, the 'Guide to Interpretation of Results' has been subsequently used as training material for laboratory members new to interpretation of aneuploidy results with our in-house assay.

Although the Instructions for Use refer to using POP6 on a 3100, we did not find it necessary to vary from the instrument's default parameters to run with POP7 on a 3130. Given that we had only recently had our analysis software upgraded from Genotyper to GeneMapper, the 'Analysis Procedure, GeneMapper 3.7' was helpful in setting up the analysis software using the Panel Manager data downloaded from the Tepnel website. Although we could have inputted the data ourselves, this undoubtedly saved time, and a minor problem that we encountered was quickly resolved by very helpful Technical Support staff at Tepnel. Among the comprehensive supporting materials, the nicely designed spreadsheet was also easy to use for the analysis of this trial data, although we may prefer to design one tailored to our own requirements for routine use in the lab.

We were very happy with the quality of the data obtained. Results were consistent with results from our 'in-house' assay (which uses a different set of markers, although some markers are common to both). The only slight exception was with a few of samples thought to have a primer site polymorphism with D21S1411 on our 'in house' assay, which is performed at a lower annealing temperature of 55°C. The allele previously exhibiting reduced amplification at 55°C showed almost no amplification under QST*R conditions at 59°C. While the marker appeared uninformative, the presence of a very small second peak may potentially raise concerns about mosaicism. We occasionally perform additional analysis for a particular marker, for example investigation of a primer site polymorphism or submicroscopic duplication, as a single marker assay. A single marker option would not be available if we switched to QST*R.

We do feel that QST*R represents a high quality product. It is an attractive option to purchase a ready made kit with the quality assurance of a CE mark, instead of making up reaction mixes ourselves, and it would have been particularly attractive had we not already developed and validated our own 'in

house' rapid aneuploidy assay. In practical terms, it would be very simple to replace our existing assay with QST*R. There are not however any significant advantages to doing so, other than removing the need to make up our own reaction mixes. The major disadvantage though for us, is the cost. We are required to screen all samples for sex chromosome aneuploidy as well as trisomy 13, 18 and 21, and so would have to buy both kits. Given that we screen over 1200 samples per annum (plus controls), any increase in consumable costs would have a significant impact on a very tight budget. As consumable cost associated with DNA extraction would remain constant, the only saving would be the relatively low cost of fluorescent primers and Qiagen multiplex buffer, plus a small amount of staff time involved in preparing reaction mixes. Even with a substantial discount from the list price, our budget would simply not support the massive increase in consumable costs involved in purchasing QST*R.

It is disappointing that we could not consider purchasing QST*R, as apart from the high cost, we were otherwise very impressed with the product. We would recommend the QST*R to anyone for whom budgets are less of an issue.

3.3.2 Edinburgh Molecular Genetics

The QST*R kit for the detection of aneuploidy of chromosomes 21, 18 and 13 was a pleasure to evaluate and performed very well in our hands. Tepnel's commercial rivals would do well to emulate the clear protocols, the easy to use reagents and the well thought out data analysis tools. In Edinburgh our own primary multiplex contains most of the microsatellite markers shrewdly selected by Tepnel and so we had very few surprises when it came to data comparisons. QST*R, however, contains 15 markers to our assay's 12 and the judicious choices for the extra markers reduces the need for repeat tests yet further. Any secondary amplifications for low heterozygosity or ambiguous peak ratios is reduced to a minimum. The QST*R kit would suit our stringent contracted turnaround times well. The batch we tested was robust and coped extremely well with the usual range of cell densities seen in the amniotic fluid specimens DNA specimens prepared using InstaGene Matrix (BIORAD).

We had little difficulty adapting existing spreadsheets and macros to analyse the data but also felt that Tepnel's own tools were very good and their on line information remains excellent. The guide they provide for the interpretation of results is a model of its kind and we felt would be hard to fault. The technical support and sales staff are also receptive and helpful which again sets this company apart from some of its rivals.

We used the same sequencer (ABI 3100) for comparing our own reagent batch with the QST*R kit. We found our own reagents behaved better for markers with larger amplicon sizes. The peak widths were wider and the trailing ladder was more pronounced in the Tepnel electropherograms. The relative peak heights for the various markers were similar to our own and we felt the kit had been expertly composed and balanced. It was reassuring to see the kit also struggled to maintain peak area ratios for marker D18S386 for the fairly frequent allele size differences of >15 repeats. It was also good to see that many of the known sequence variants likely to lie under primer positions and compromise quantitative amplification had been expertly side stepped.

Where our own reagents performed significantly better was with DNA specimens prepared using other extraction methods. Samples extracted using the EZ1 DNA tissue kit (QIAGEN) in conjunction with the BioRobot EZ1 Workstation (QIAGEN) are now routinely used in our lab. The rapidity of the protocol and the reliability and reproducibility of DNA qualities for the typical range of cell densities is better suited to the turnaround times we are now contracted to deliver. The QST*R kit did not cope as well with DNA prepared in this way. The amplification was less good and the baseline noise was markedly increased. We recognise that appropriate adaptation of protocols was not possible with the limited number of reactions used for this evaluation and that a protocol more suited to this extraction type might give better results. This is an area where our own reagents proved more universally suitable.

We liked the QST*R kit and felt it was a high quality new "stable mate" for existing Tepnel assays. The advantages both of using a recognised approved reagent and no longer needing to balance complex multiplex PCRs on a regular basis would be ideal for our service were it not for the cost differential. The loss of individually matched secondary tests in clued single marker amplifications where required might be another problem. There is little doubt that apart from the tripling of reagent costs using this assay would entail that it would meet the needs of our service.

3.3.3 Aberdeen Molecular Laboratory

In addition to submitting DNA samples to NGRL (Wessex) we also conducted a small scale experiment to compare QST*R with our in-house QF-PCR kit.

Comparison was made with regard to:

- Number of informative markers for each chromosome
- Range of allele area ratios
- Ease of use

Findings:

- The QST*R kit was easy to use and the instructions were easy to follow
- For the samples tested the QST*R kit gave the most informative results
- Based on these results we have decided to conduct further validation of the QST*R kit

Further validation:

We are now in the process of a comparative validation trial of the QST*R kit with a larger number of samples (~100).

*Future use of QST*R:*

If our validation trial goes well we would like to use QST*R for diagnostic QF-PCR testing within our laboratory. The cost is a consideration, especially as we estimate it will be 2-3 times more expensive than our in-house kit. This will have to be considered when all of the data is available. We are in no doubt that a commercially prepared kit is the way we want to go.

3.4 Costings

Current list prices can be obtained directly from ELUCIGENE or their distributors. Contact details can be found at www.elucigene.com.

4. CONCLUSIONS

ELUCIGENE QST*R™ is technically easy to use, CE marked and therefore IVD compliant. It fulfils the typical requirements of a rapid prenatal test; the assay was accurate and no false positive results were obtained, the kits coped well with variable DNA quality, the results obtained were unambiguous and the failure rate was low. The manual supplied with the kit was excellent and the instructions and advice were easy to follow.

Over 95% of tests could have been reported without follow up studies being required. Use of the extra marker sets resolved single marker inconclusive allele ratios and provided extra informativity in all cases that required follow up. QST*R results were 100% consistent with the sample karyotype for the retrospectively collected DNA tissue samples and amniotic fluid samples from NGRL (Wessex). Follow up studies were not undertaken on the samples submitted from other laboratories but for the samples that did not require additional follow up studies the QST*R results were 100% consistent with the 'in house' QF-PCR results.

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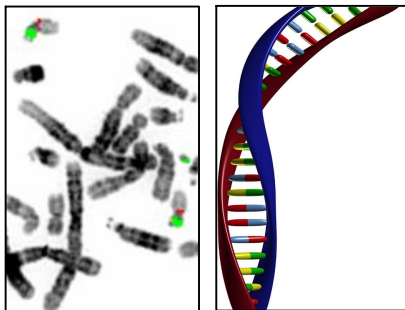
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