**Background**

- The gold standard diagnostic method for prenatal detection of chromosomal aneuploidy is currently karyotyping cultured foetal cells obtained from amniotic fluid (AF) or chorionic villus sampling (CVS).
- Karyotyping is capable of detecting all cytogenetic abnormalities but the average UK reporting times are 13.5 days for AF and 14.8 days for CVS.
- **NGRL (Wessex)** has established a collaboration with six network laboratories to evaluate two CE marked in vitro diagnostic test kits for the analysis of aneuploidy: **ELUCIGENE QST*R**™ (Tepnel Diagnostics) and **Aneufast**™ (Genomed Ltd).
- **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 available for the detection of sex chromosome aneuploidy. Aneufast, for the combined prenatal diagnosis of trisomy 13, 18, 21 & sex chromosome aneuploidy, was developed in conjunction with the Department of Medical Genetics in Barcelona.
- **Initially, 240 amniotic fluid (AF) DNA samples and 87 retrospectively collected aneuploid tissue DNA samples were tested by NGRL (Wessex) using both kits. An additional 168 prenatal DNA samples were 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 PCR products were analysed with an ABI 3100 and 3130 using Genotyper v3.7 and GeneMapper v3.7 respectively.**
- **Kits 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.**

### ELUCIGENE QST*R™

**Kit design**

The five Elucigen QST*R kits are CE marked and therefore compliant with the **In Vitro Medical Devices Directive (98/79/EC):**

- **QST*R:** Single tube multiplex used for the routine detection of the three viable autosomal trisomies: trisomy 13, trisomy 18 & trisomy 21
- **QST*R-XY:** Single tube multiplex used for the evaluation of sex chromosome status
- **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.

**Preliminary Results**

Retrospectively collected tissue DNA samples (n=87) from: normal controls (n=24), trisomy 18 (n=13), trisomy 13 (n=12), trisomy 21 (n=19) and sex chromosome aneuploidy (n=19) and DNA samples from 1ml amniotic fluid samples (n=342), normal (n=225), trisomy 13 (n=2), trisomy 21 (n=3), triploid (n=2), sex chromosome aneuploidy (n=2) were tested using ELUCIGENE QST*R™. For this evaluation, DNA was extracted from amniotic fluid using either InstaGene Matrix (BIORAD) or the EZ1 DNA tissue kit (QIAGEN) in conjunction with the BioRobot EZ1 Workstation (QIAGEN). No chorionic villus samples were evaluated. Examples of QST*R Genotyper traces are shown in Figure 1a and b and the results of testing the samples using an ABI3100 and analysing with Genotyper v3.7 are shown in table 1. Data from the samples collected from other Network laboratories (n=168) will be included in the **NGR (Wessex) report that will be distributed in November 2006.**

**Conclusions**

QST*R was technically easy to use and is IVD compliant. The manual was easy to follow and the analysis conditions and parameters supplied worked well and did not require any further optimisation.

All QST*R results were consistent with the sample karyotype

Samples which required single marker repeats had either been in storage for a year or were of poor quality when assessed using a quality specific PCR.

### Aneufast™

**Kit design**

The Aneufast™ QF-PCR kit is CE marked and therefore compliant with the **In Vitro Medical Devices Directive (98/79/EC):** It contains six multiplex marker sets of short tandem repeats (STRs) that can be used for amplification of selected microsatellites and the Amelogenin-SRY. Aneufast is comprised of two multiplex QF-PCR sets (S1and S2) which can be used for the diagnosis of trisomy 13, 18, 21 & sex chromosome aneuploidies. S1 and S2 are combined and analysed in a single electrophoresis. Four chromosome-specific marker sets (M21, M13, M18 and MXY) are also supplied which can be used for extended testing when a definitive result is not obtained using S1 and S2.

**Preliminary Results**

Data from the Aneufast evaluation is still under analysis. Preliminary findings have shown that tube S1 amplifies more strongly than S2 when using the conditions outlined in the Aneufast manual. This makes it difficult to analyse the two multiplexes in a single electrophoresis. Figure 2 shows an example of a Genotyper plot for a normal female sample where markers S1 and S2 have been combined.

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