Rapid methods to detect aneuploidy

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Introduction

The aim of this document is to provide a brief outline summary of the current status, advantages and disadvantages of molecular methods to detect chromosomal aneuploidy. All the techniques below are capable of delivering results within 1-3 working days following receipt of amniocentesis or chorionic villus samples, and are viewed as a prelude to, rather than a replacement of, full karyotype analysis following an invasive prenatal procedure. General problems for the detection of aneuploidy include maternal cell contamination (MCC), low level mosaicism, triploidy and sample quality. Poor sample quality leading to failures and very low level mosaicism is assumed to affect all techniques and are not considered.

Although periodically there is great excitement about the prospect for non-invasive prenatal diagnosis by analysis of foetal cells or DNA in maternal peripheral blood or urine there is, as yet, no technique likely to enter routine practice in the near future. However a number of avenues continue to be explored.

Methods currently available

Quantitative-fluoresence PCR (QF-PCR)
Method. Relative quantitation of microsatellite alleles. Typically, multiplex PCR is performed with fluorescently labelled primers and products fractionated and quantified on a genetic analyser.
Advantages. Extensively validated. High throughput. Detects MCC in the great majority of cases without the need for a maternal sample. Detects triploidy and mosaicism (down to a level of approximately 15% abnormal cells). Requires only small amounts of DNA.
Disadvantages. Although QF-PCR works very well in a high throughput format, it is much more troublesome and less cost effective if samples cannot be batched. Relatively low throughput is typical of most regional genetics laboratories.
Comments. A very small proportion of cases (0.05%) are uninformative. Despite the extensive use of QF-PCR in several centres, the methodology is not yet standardised, although Best Practice guidelines have been drawn up recently by the cytogenetic and molecular professional bodies. A CE marked kit manufactured by CyberGene is due to be launched shortly in the UK but currently it is not known how well this will perform and, in particular, if it will help with the problem of throughput. NGRL (W) and other centres will be testing the kit as soon as it is available.

Interphase fluoresence *in situ* hybridization (FISH)
Method. Hybridisation of fluorescently labelled chromosome-specific probes to uncultured samples. Manual enumeration of number of hybridising signals in 20-50 (or more) nuclei.
Advantages. Extensively validated. Robust. Detects mosaicism and triploidy. Kits available for diagnostic use.
Disadvantages. Low throughput, expensive due to reagents and labour costs.
Comments. Widely used but only really practical when numbers are relatively small. In contrast to DNA based techniques, MCC is not considered to be a serious problem since any contaminating maternal blood cells are either morphologically distinguishable from foetal cells, or are present in aggregates that are not analysed. Throughput would theoretically be increased if reliable automated spot counting systems could be adapted for prenatal diagnosis, but this is technically difficult.

Multiplex ligation dependent amplification (MLPA)
Method. Hybridisation of multiple tagged oligonucleotide probe pairs to defined genomic regions, ligation of correctly hybridised probe pairs, amplification using common fluorescent primers, products fractionated and quantified on a genetic analyser.
Advantages. Although recently introduced, the technology is perceived as robust and widely applied to detect copy number variation. In principle, will work well in both high and low throughput formats. Available in kit form (although not formally approved for diagnostic use).
Disadvantages. Not formally validated (but already is use in some centres). Overnight hybridisation step means that results cannot currently be obtained within one day. Cannot detect triploidy. With the current design of probes MCC will not be detected for a female foetus unless a maternal sample is also analysed (by a separate assay alongside the test sample).
Comments. Although the technique is likely to have a very low false positive and false negative rate several performance characteristics need to be established, eg. (i) failure rate (it is known that the assay is particularly sensitive to DNA quality), (ii) sensitivity with which mosaicism can be detected (theoretically may be less sensitive for detection of low level mosaicism than QF-PCR) and (iii) degree to which MCC can be identified. Detailed evaluation of MLPA by Dutch laboratories plus NGRL (W) due to start soon.

Methods under development

Paralagous gene quantification
Method. Co-amplification with a single primer pair of paralogous sequences that are present on the chromosome of interest and one other autosome. Determination of the relative amount of the two sequences by quantitative analysis of small sequence differences between the two paralogues by Pyrosequencing™ (or another method that provides accurate quantitation of SNPs).
Comments. Internally controlled technique that could be further developed, potentially producing a robust assay that would work well in both low and high throughout formats. Despite encouraging results published recently on relatively small numbers of samples, NGRL (W) has found that detection of trisomy 13 and trisomy 18 is unreliable, although detection of trisomy 21 worked well. The current problems may

be due to paralogue sequence instability or the presence of additional uncharacterised genomic paralogues.

Real time PCR
Method. Measurement of amounts of PCR products at each round of amplification by fluoresence resonance energy transfer and appropriately labelled hydrolysis (TaqMan™) or hybridisation (LightCycler™) probes. Quantitation is performed relative to external or internal standards on a dedicated real time instrument.
Comments. Although proof on principle analyses have been published, routine detection of 3:2 ratios is probably beyond the limit of resolution of the technology. Could in principle be adapted to the quantitative detection of SNPs (see below).

Multiplex amplifiable probe hybridisation (MAPH)
Method. Hybridisation of multiple tagged short probes to genomic DNA, quantitative recovery, amplification using common fluorescent primers, products fractionated and quantified on a genetic analyser
Comments. Proof of principle established, advantages and disadvantages similar to MLPA, currently rather cumbersome but under further development.

Microarray comparative genomic hybridisation (CGH)
Method. Hybridisation of differentially fluorescently labelled genomic and control DNA to genomic DNA clones gridded out on slide arrays. Signal intensities read by a scanner
Comments. Currently very expensive, but could be multiplexed by including several miniarrays on a single slide. Proof on principle established. Probably likely to be used as a high resolution whole genome scan or as a targeted prenatal chip covering common microdeletions and microduplications rather than specifically for aneuploidy detection.

Quantitative single nucleotide polymorphism analysis (Q-SNP)
Method. Pyrosequencing™, mass spectrometry, SNP arrays, eg Affymetrix™, Luminex™.
Comments. In principle, any technique that can provide robust quantitative SNP information in a multiplex fashion could be developed to provide an assay with all the current advantages of QF-PCR, including detection of MCC. Techniques such as Pyrosequencing™ should work well in low to medium throughput platforms, mass spectrometry may be best for high throughput. However these assays need development.

Summary

Currently, QF-PCR has a number of significant advantages over other techniques, provided that throughput is relatively high. For low throughput, FISH remains an alternative and MLPA, possibly in combination with a separate MCC test, is likely to emerge shortly as a robust alternative. In the future, it is possible that rapid aneuploidy analysis may be performed routinely by high throughput quantitative SNP detection techniques, such as mass spectrometry.

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