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Review

Rapid and simple prenatal diagnosis of common chromosome disorders: advantages and disadvantages of the molecular methods FISH and QF–PCR

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Molecular techniques have been developed for prenatal diagnosis of the most common chromosome disorders (trisomies 21, 13, 18 and sex chromosome aneuploidies) where results are available within a day or two. This involves fluorescence *in situ* hybridization (FISH) and microscopy analysis of fetal cells or quantitative fluorescence polymerase chain reaction (QF–PCR) on fetal DNA. Guidance is provided on the technological pitfalls in setting up and running these methods. Both methods are reliable, and the risk for misdiagnosis is low, although slightly higher for FISH. FISH is also more labour intensive than QF–PCR, the latter lending itself more easily to automation. These tests have been used as a preamble to full chromosome analysis by microscopy. However, there is a trend to apply the tests as 'stand-alone' tests for women who are at relatively low risk of having a baby with a chromosome disorder, in particular that associated with advanced age or results of maternal serum screening programmes. These women comprise the majority of those currently offered prenatal diagnosis with respect to fetal chromosome disorders and if introduced on a larger scale, the use of FISH and QF–PCR would lead to substantial economical savings. The implication, on the other hand, is that around one in 500 to one in 1000 cases with a mentally and/or physically disabling chromosome disorder would remain undiagnosed.

Prenatal diagnosis with a view to identifying fetal genetic disorders started in the early 1970s. Since its inception, the most common reason for prenatal diagnosis is increased risk of having a child with trisomy 21 Down syndrome. This risk is dependent on maternal age, and is also assessed by maternal serum screening programmes and fetal ultrasonography (nuchal translucency). Other indications for prenatal diagnosis of chromosome disorders include additional structural fetal abnormalities detected by ultrasonography, a previous child with a chromosome disorder or either parent being a carrier of a chromosomal rearrangement.

Over the years prenatal diagnosis has become increasingly common; for example, to date it involves around 1 in 20 pregnant women in the UK. Fetal cells for chromosome diagnosis are obtained either by amniocentesis (usually at about 15 weeks of gestation) or chorionic villus sampling (at about 9–11 weeks of gestation). Both these procedures are invasive and carry an associated risk for induced abortion in approximately 1 in 100 to 1 in 200 women tested. Traditionally, chromosome diagnosis is accomplished by karyotyping, that is, analysis of chromosomes by microscopy followed by the lining up of each chromosome pair (Fig. 1).

Karyotyping, most often by Giemsa banding (G-banding) is performed on fetal cells at the metaphase stage of the cell cycle, when chromosomes are optimally condensed. Amniotic fluid samples obtained by amniocentesis do not contain any fetal cells in division and have to be grown *in vitro* to obtain cells at the metaphase stage. In chorionic villus samples (CVSs), some fetal cells are spontaneously dividing and cells at metaphase can be used. However, the resolution (of special importance for the detection of structural abnormalities) may be quite low, as these spontaneously dividing cells have more condensed chromosomes than those obtained after cell culture *in vitro*. Another disadvantage of CVSs is the common occurrence of confined placental mosaicism, the occurrence of a proportion of aberrant cells that do not represent the chromosome status of the fetus. These aberrant cells when originating in cytotrophoblasts may be selected against during cell culture *in vitro*. Therefore, cell culture is usually performed as a follow-up to any direct chromosome diagnosis on CVSs.

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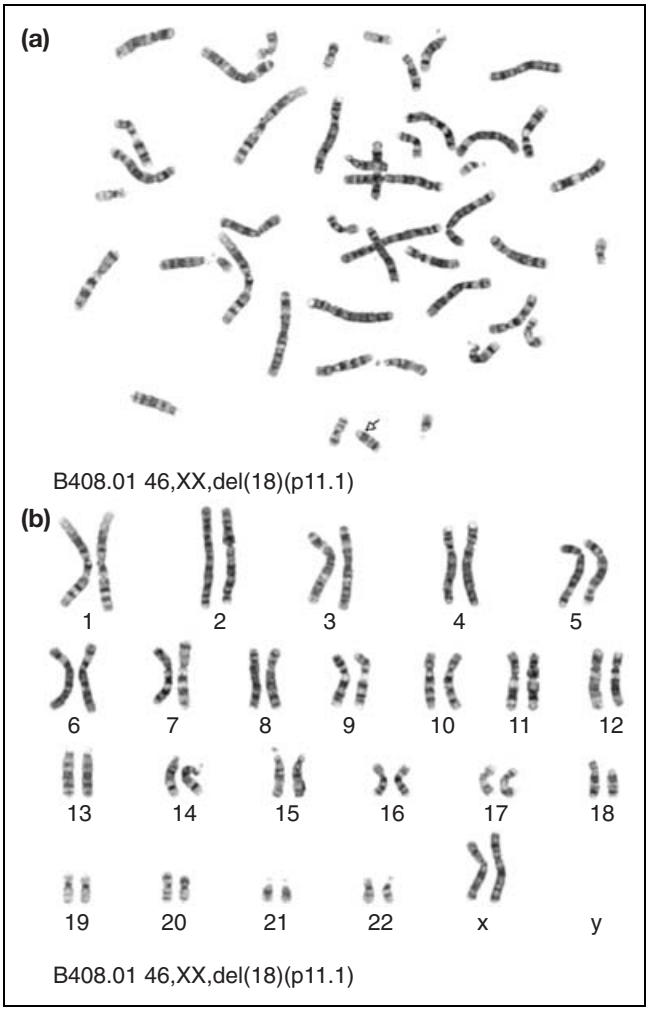

Fig. 1. G-banded chromosomes from a female with an 18p deletion and the XX sex chromosome constitution. The deletion (arrow) involves about 25 Mb of DNA. (a) Metaphase plate and (b) the corresponding karyotype.

During the last three decades, improved technology for prenatal diagnosis by karyotyping has mainly involved devising methods for obtaining less condensed chromosomes and for reducing culture time.

The presence of a larger number of bands on longer chromosomes implies that subtler structural chromosome abnormalities may be detected. However, it is important to note that even at high resolution G-banding, deletions or duplications < 5 Mb will usually remain undetected. The implication is that a proportion of chromosome abnormalities, which may be associated with physical and mental disability, will not be routinely diagnosed, even with this 'gold standard' test.

In the early days, it took around 3–4 weeks of culture of amniocytes *in vitro* to obtain enough cells in division for karyotyping but substantial reduction in culture time of both amniocytes and chorionic villus cells has been achieved more recently. For example, from 1987 to 1998, the average reporting time in the UK decreased from 20.2 to 13.8 days for amniotic fluid samples and from 21.3 to 14.5 days for CVSs (Waters and Waters, 1999). Some laboratories have been able to reduce the culture time *in vitro* even further (for example, see Miller and Peakman, 1999).

It is recognized that long waiting times for results may cause much psychological suffering and this has been one of the main reasons for the introduction of molecular methods for prenatal diagnosis of common chromosome disorders. This type of approach does not require cell culture and reports can routinely be issued within 1–2 days.

Provision of rapid and simple detection of common fetal chromosome disorders

The two most common types of molecular method for prenatal diagnosis of chromosome disorders are fluorescence *in situ* hybridization (FISH) and the quantitative fluorescence polymerase chain reaction (QF–PCR). Both methods are now used routinely for rapid and simple diagnosis of aneuploidy (numerical chromosome abnormalities), including in particular trisomy 21, 13 and 18 (giving rise to the Down, Patau and Edward syndromes, respectively) and sex chromosome abnormalities (associated with the XXY-Klinefelter, XYY, XXX and X-Turner syndromes). The disorders listed above are the most common. Overall they comprise 70–80% of karyotyped abnormalities associated with physical–mental handicap. However, they constitute 99.8–99.9% in low-risk pregnancies, in which women are tested because of maternal age, maternal serum or ultrasonographic screening for fetal nuchal translucency (for example, see Ward *et al*., 1993; Evans *et al*., 1999; Pergament *et al*., 2000; Thein *et al*., 2000; Grimshaw *et al*., 2003). Notably, there is some variation among studies, and a much higher rate of residual abnormality has been identified in a recent study by Homer *et al.* (2003).

FISH and QF–PCR involve DNA fluorescence but in different ways

FISH and QF–PCR both make use of fluorescence tagging of DNA, but chromosome diagnosis is accomplished by each in very different ways.

FISH

FISH involves hybridization of selected chromosome-specific DNA sequences that have been labelled with fluorescent dyes to chromosome preparations. The fluorescently labelled sequences stick to the corresponding DNA of the chromosomes and can be visualized under the microscope (for review, see Lichter and Cremer,

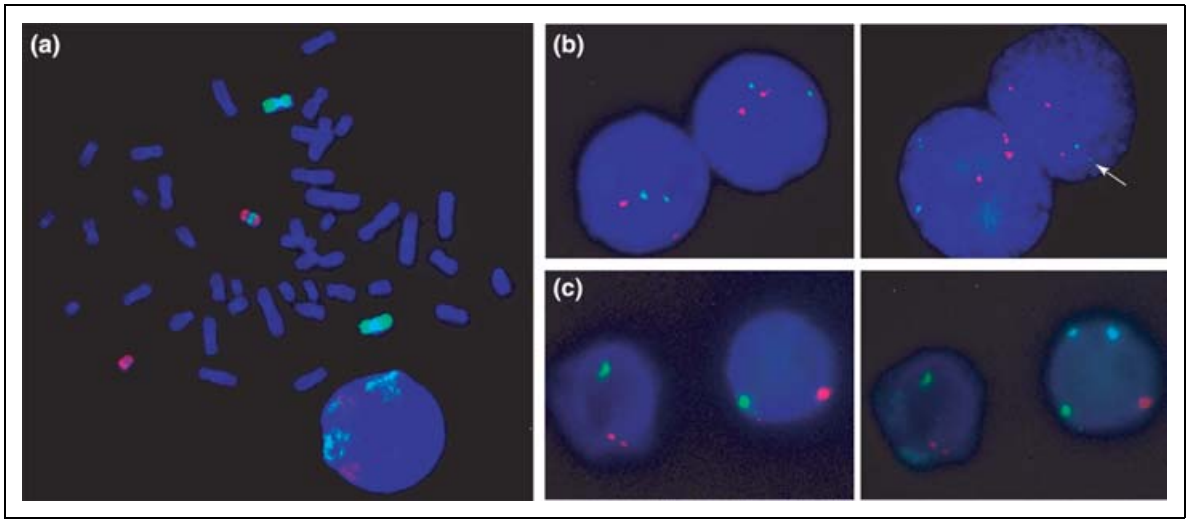

Fig. 2. Fluorescence *in situ* hybridization (FISH) images of metaphase and interphase nuclei. (a) Metaphase plate and interphase nuclei from a female carrier of an insertion of a segment of chromosome 20 into chromosome 9. Labelling with whole chromosome FISH probes specific for chromosome 9 (green) and chromosome 20 (red). Note that, in the metaphase, the chromosomes are distinctive but, in the interphase nuclei, they appear as fuzzy clouds. (b) FISH images of interphase nuclei from uncultured amniocytes hybridized with probes specific for chromosomes 21 and 13. In the normal case (left), there are two clear red signals corresponding to the two copies of chromosome 21 and two green signals corresponding to chromosome 13. In the trisomy 21 case (right), there are three clear red signals in both interphase nuclei, indicating the presence of an extra copy of chromosome 21. Note the split red signal, which could cause problems in interpreting the number of chromosomes present. By convention, a score as a single chromosome spot is assigned if the distance between spots is smaller than their size in any focal plane. Note also that one of the green signals is much smaller than the other (arrow), in this case indicating that the chromosome is lying in a different focal plane. Thus, it is very important to focus up and down when analysing interphase nuclei for aneuploidy diagnosis by microscopy. (c) FISH images of interphase nuclei from uncultured amniocytes from a normal male, hybridized with chromosome 18, X and Y probes. The image on the left shows the X- and Y-chromosomes in green and red, respectively, indicating a normal XY male. The image on the right shows the same nuclei with the chromosome 18 signals in aqua. Note that, as the left nucleus is out of focus, the Y signal appears split and the chromosome 18 spots are very diffuse and hardly identifiable.

2001). The first developed probes were derived from DNA of flow-sorted whole chromosomes and used for prenatal diagnosis of trisomy 21, 13 or 18 by Kuo *et al* (1991) on uncultured amniotic fluid cell nuclei. The drawback of this approach is that the signals obtained may be quite diffuse, as chromosomes at interphase of the cell cycle often appear much less condensed than those at metaphase, if standard fixation technologies are used (Fig. 2a). For aneuploidy diagnosis, FISH with smaller probes is advantageous, as signals appear as more distinct dots. Normal samples are expected to show two dots per cell nucleus, whereas those that are trisomic will show three dots (Fig. 2b,c). Initially, a variety of such probes was developed in research laboratories. More recently, probe sets have been produced commercially (Vysis) and are applied in batches, highlighting chromosomes 13 and 21 in one hybridization and 18 plus X and Y in another.

It should be noted that whole chromosome paints may in fact be successfully used, measuring the fluorescence ratio between a target chromosome in relation to a standard of similar size, not likely to be represented as a trisomy at the time of prenatal diagnosis (Truong *et al*., 2003).

QF–PCR

The most common type of QF–PCR involves the amplification of chromosome-specific, repeated DNA sequences known as small (short) tandem repeats (STRs). STRs are stable and polymorphic, that is, they vary in length between subjects, depending on the number of times the tri-, tetra- or penta-nucleotides are repeated. The sample DNA is amplified by PCR using fluorescent primers so that products can be visualized and quantified as peak areas of the respective repeat lengths using an automated DNA sequencer with the Gene-Scan software (Fig. 3). DNA amplified from normal subjects who are heterozygous (have alleles of different lengths) is expected to show two peaks with the same area. DNA amplified from subjects who are trisomic will exhibit either an extra peak (being triallelic) with the same area, or only two peaks (being diallelic), one of them twice as large as the other. By convention (agreed at a Symposium

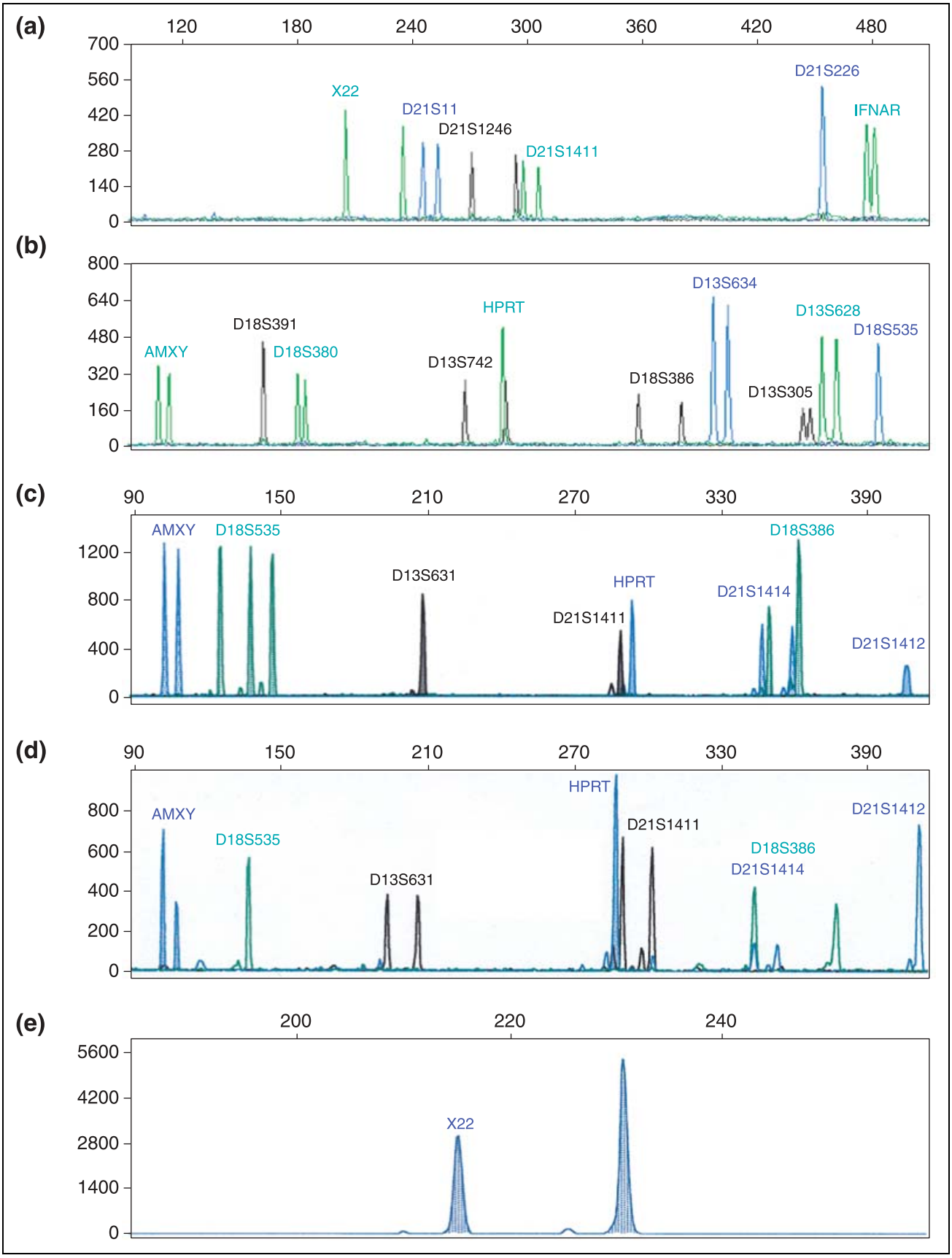

Fig. 3. For caption see facing page.

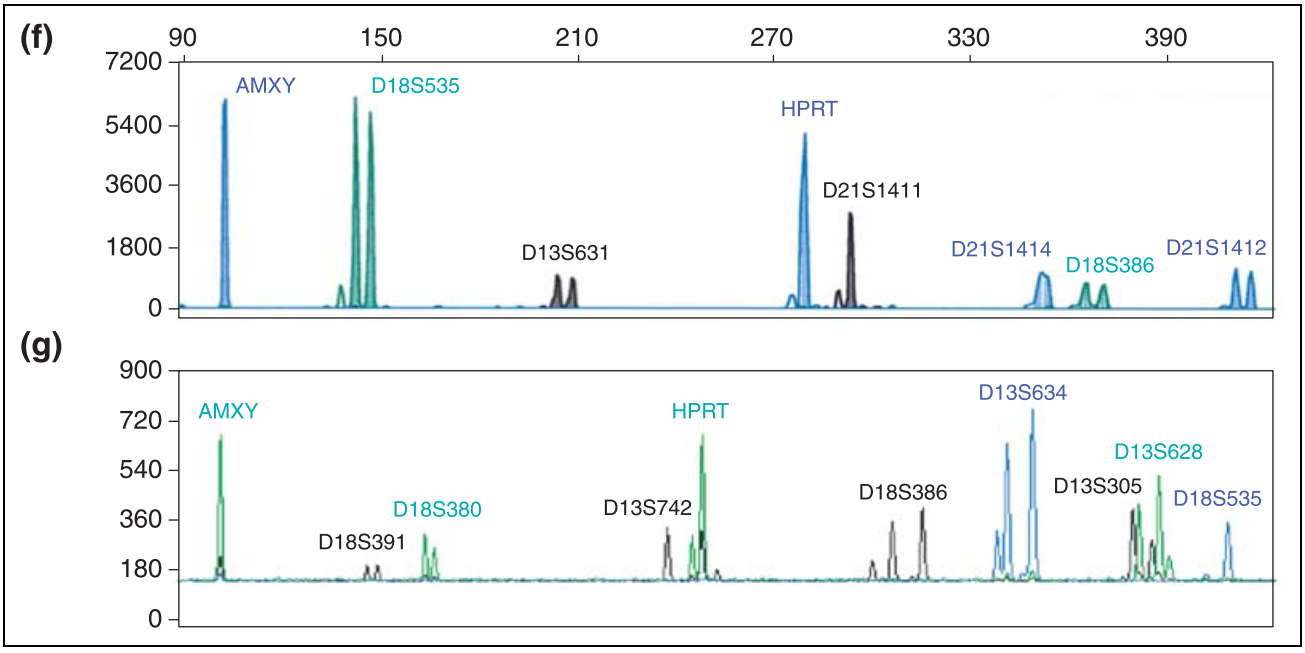

Fig. 3. Electrophoretograms of QF–PCR amplifications. The *x*-axis shows the length of the PCR products in base pairs and the *y*-axis shows the fluorescence intensity in arbitrary units. (a) DNA sample from a normal subject amplified with X22 (green), and 5 markers for chromosome 21: D21S11 (blue), D21S1246 (black), D21S1411 (green), D21S226 (blue) and IFNAR (green). Except for D21S226, which is homozygous, all the 21 markers are heterozygous, with two peaks of nearly 1:1 ratio. (b) DNA sample from the same subject amplified with four markers each for chromosomes 13 and 18 and AMXY and HPRT for the sex chromosomes. AMXY (green) indicates a 46,XY male, HPRT (green) is uninformative showing homozygosity, D18S391 (black) is also uninformative showing homozygosity, D18S380 (green) is informative showing heterozygosity with two peaks of nearly 1:1 ratio, D18S386 (black) is also informative showing heterozygosity with two peaks of nearly 1:1 ratio, D18S535 (blue) and D13S742 (black) are uninformative showing homozygosity, whereas D13S634 (blue), D13S305 (black) and D13S628 (green) are informative showing heterozygosity with two peaks of nearly 1:1 ratios. (c) DNA sample from a trisomy 18 case amplified with AMXY (blue), D18S535 (green), D13S631 (black), HPRT (blue), D21S1411 (blue), D18S386 (green) and D21S1412 (blue) in a single multiplex reaction. D18S535 displays triallelic trisomic pattern with three peaks of approximately 1:1:1 ratio, whereas D18S386 shows the diallelic ratio of 0.56 within the trisomic range. (d) DNA sample from a suspected 47,XXY (Klinefelter syndrome) case amplified with the same markers as in (c). A modified version of the AMXY is used, showing a 2:1 ratio for X:Y indicating the XXY constitution. As HPRT is homozygous, no definite conclusion can be derived unless the sample is re-amplified with additional X- and Y-chromosome-specific markers, showing heterozygosity. (e) Same sample as in (d), amplified with the X22 marker, displaying two peaks with a 2:1 ratio confirming the XXY status. (f) Example of suspected 45,X (Turner syndrome). DNA amplified with the markers as in (c), showing only one peak for AMXY and one for HPRT, hence making it difficult to differentiate between a normal female homozygous for HPRT and a 45,X Turner case. Therefore, the DNA of this case has to be amplified with additional X- and Y-chromosome-specific markers for reliable diagnosis. (g) Example of maternal contamination showing multiple triallelic peaks outside the 1:1:1 ratio, precluding reliable diagnosis on fetal aneuploidy.

in Austria in 2001 arranged by Barbara Pertl), samples that are diallelic, containing two peaks with area ratios of the shorter and the longer allele within the range 0.8–1.4, are considered to be normal. In contrast, diallelic samples with ratios <0.65 or >1.8, are considered to be trisomic (for examples, see Fig.3a–c).

STRs that are highly polymorphic have been commonly used as markers for QF–PCR. However, single nucleotide polymorphisms (SNPs), which are much more frequent in the human genome, present an alternative option, likely to become more commonly applied in future. In addition, unique DNA sequences may be applied, for example using the novel multiplex ligation-dependent probe amplification (MLPA) technique (Schouten *et al*., 2002).

FISH requires larger samples and is more labour intensive than QF–PCR

FISH

Aneuploidy diagnosis by FISH is performed on preparations made from around 1.0–1.5 ml of amniotic fluid. After centrifugation, cells in the pellet are fixed and placed on microscopy slides and hybridized with FISH probes (Klinger *et al*., 1992). Samples may be handled in batches but the process is still quite time consuming (Grimshaw *et al*., 2003). Some reduction in time may be achieved by the use of automatic harvesters (for example, Hamilton). The most time-consuming part of the interphase FISH procedure concerns fluorescence microscopy, and involves spot counting of 25–50 nuclei with

Table 1. Apparently false positive and false negative fluorescence *in situ* hybridization (FISH) signals per 1000 interphase nuclei in amniotic fluid samples diagnosed by karyotyping as normal (non-mosaic) 46,XX or 46,XY

	X	Y	21	13	18
False positive	7	0.6	20	12	17
False negative	6	2	30	32	39

A total of 353 cases was analysed comprising in total 18 060 interphase nuclei.

respect to chromosomes 21 and 13 in one batch, and chromosomes 18, X and Y in another. This is expected to take about 30 min per sample. A relatively large number of cells is analysed, as a proportion of normal nuclei may show either a missing (false negative) or an extra (false positive) signal (Table 1). Some laboratories are routinely counting around 100 cells in order to increase the chance of detecting constitutional mosaicism (see below). Several firms are developing image analysis software for automated spot counting (for example, Applied Imaging, Bioview, Imstar and Metasystems) but as yet there are no published reports on their accuracy or efficiency in terms of labour saved.

QF–PCR

QF–PCR can accommodate smaller sized samples than are required for FISH for prenatal aneuploidy diagnosis. Routinely, DNA is extracted in batches from about 0.5–1.0 ml amniotic fluid per case, taken at about 15 weeks, followed by PCR amplification and gel electrophoresis on a DNA sequencer. An alternative option involves sampling the exo-coelomic fluid, which can take place much earlier, at 5 weeks (Jauniaux *et al*., 2003). The analysis when carried out by the Gene-Scan software on an automated DNA sequencer (for example, Applied Biosystems) is expected to take about 5 min per sample. QF–PCR is more amenable to automation than FISH, and a large number of samples can be handled simultaneously, allowing substantial economy of scale (Grimshaw *et al*., 2003).

The markers, specific for chromosomes 21, 13, 18, X and Y that have been used for QF–PCR, together with their heterozygosity, are exemplified (Table 2). Original studies applied one marker at a time but several more recent reports describe the development of multiplex assays in which 4–12 markers are co-amplified in different combinations (Table 3). Most often 1–3 multiplex assays are used for aneuploidy diagnosis with a minimum of two informative markers per chromosome required for confident diagnosis. Samples that are uninformative (or suspected to be abnormal) may be re-tested, using additional markers as a back-up.

In our view, the most taxing part of setting up a QF–PCR service for prenatal interphase diagnosis concerns optimization of the primers to be included in any one multiplex reaction. To date, there are no commercial STR multiplex primer kits available.

The risk for misdiagnosis by either FISH or QF–PCR is relatively small

FISH

There are now a large number of reports in the literature highlighting the efficacy of rapid prenatal aneuploidy diagnosis, using FISH probes on interphase nuclei. The experience gained so far with respect to the proportion of cases that are informative, and the risk for misdiagnosis, is summarized (Table 4).

Some DNA sequences may be shared in common between different centromeres, and the initial FISH probes developed for certain centromeres cross-hybridized to others leading to diagnostic failures. The development of chromosome-specific probes, using unique DNA sequences has, to a large extent, eliminated this type of problem. Remaining diagnostic problems seen with the commercially available probe sets for chromosomes 21, 13, 18, X and Y include, in particular, constitutional heteromorphisms leading to false positive or false negative results (Table 5).

The first prospective FISH study on interphase amniocytes, using probes for single copy-like signals of chromosomes 21, 18, 13, X and Y appeared more than a decade ago (Klinger *et al*., 1992). This seminal investigation was followed only a year later by another (and much larger) prospective study involving 4500 samples, showing 90.2% informativeness when using region-specific probes for the same chromosomes (Ward *et al*., 1993).

Many more recent literature reports have indicated a much higher informativeness. They also show that the risk of either over- or underdiagnosis of anueploidy for the target chromosomes is small by interphase FISH, using the 'gold standard' of karyotyping as a comparison (Table 4). One recent review summarizes data on 29 039 cases (Tepperberg *et al*., 2001). On the basis of their most recent series of 5197 informative tests, using the Aneuvysion probe set (Vysis), the authors predict a risk for a false positive result to be less than 1 in 30 000 cases and that of a false negative to be less than 1 in 4000. However, not all centres agree. Thus, for example, Weremowicz *et al*. (2001) comment that, in their experience, the sensitivity (the probability that the FISH test will accurately predict karyotypic aneuploidy) and specificity (the probability that the FISH test will accurately predict karyotypic normality) is much lower (Table 4). It should be added that some discrepancies might in fact be expected between uncultured and cultured cell populations, particularly with respect to maternal cell contamination and mosaic cell lines. The reason for this is that during cell proliferation *in vitro*,

some cell types may be preferentially selected against and thus lost for karyotyping.

QF–PCR

The first QF–PCR application involved X chromosome aneuploidy (Lubin *et al*., 1991). This was followed by investigation of the trisomies 21, 13 and 18 in a relatively small number of cases in single or multiplex PCR reactions (Mansfield, 1993; Pertl *et al*., 1994; Adinolfi *et al*., 1995). More recently, a number of reports on larger series have been published (Table 3). Several other smaller studies confirm the high reliability and reproducibility of the QF–PCR assay (Findlay *et al*., 1998; Pertl *et al*., 1999; Yang *et al*., 1999; Chen *et al*., 2000; Bili *et al*., 2002). False negative or false positive results using QF–PCR are rare, in particular when analysing autosomal chromosomes. The main problem QF–PCR has posed is when testing for sex chromosome abnormalities. When STRs specific for chromosome X are used, some samples from normal XX females may show homozygous QF–PCR patterns, indistinguishable from those produced by samples with a single X, as in Turner syndrome (Fig. 3f). Incorporating additional X-chromosome markers into the analysis will reduce the likelihood of homozygosity. Cirigliano *et al*. (2002) further suggested that the addition of an autosomal marker (such as D21S1411) as an internal control for quantification of the X-chromosome marker HPRT may solve the problem.

It is important to note that the occurrence of an extra or missing signal (or abnormal ratios) for a single marker in an otherwise normal multiplex reaction may not necessarily represent a technical artefact. There is a possibility that this could be caused by a fetal constitutional duplication or deletion of the chromosome segment, where the marker is localized. In this situation, it is advisable to check the same in DNA from parental blood samples. It may also be prudent to test adjacent markers to identify the size of the suspected duplication or deletion, which may either constitute a harmless polymorphism or be associated with mental and/or physical handicap.

Maternal cell contamination may constitute more of a problem with FISH than with QF–PCR

It has been estimated that a large proportion of amniotic fluid samples (21.4%) is contaminated with some maternal cells. However, a much lower proportion (1–2%) is macroscopically blood stained, potentially leading to false negative diagnosis of fetal aneuploidy (Winsor *et al*., 1996). For safety, many laboratories would discard any such heavily contaminated samples with respect to rapid prenatal diagnosis.

FISH

One of the disadvantages of FISH is that maternal and fetal XX cells *per se* are indistinguishable by FISH, rendering maternal cell contamination undetectable from female fetuses. This is not an uncommon reason for misdiagnosis (Table 5). However, maternal cell contamination is readily detectable with male fetuses, as a mixture of XX and XY cells are then seen. (The same is found in cases of fetal true hermaphroditism but such cases are exceedingly rare.)

QF–PCR

In contrast to the situation with FISH, maternal cell contamination is readily detected by QF–PCR amplification of STRs. A characteristic pattern with extra alleles or skewed ratios between peaks for the target chromosomes is seen (Fig. 3g). Macroscopically blood-stained samples showing such results have in the past generally been considered unsuitable for PCR diagnosis. Nevertheless, accurate diagnosis may often be achieved by careful comparison with profiles from maternal blood samples.

Constitutional fetal mosaicism remains a challenge by either method

A related problem is the diagnosis of constitutional fetal mosaicism, the occurrence of different fetal cell lines containing different numbers of chromosomes.

FISH

The sensitivity of the FISH probes is not 100% (Table 1). Therefore, a relatively large number of cells (in the order of 25–50) have to be examined, and cut-off levels for proportions of apparently normal and apparently aneuploid nuclei set (Ward *et al*., 1993; Tepperberg *et al*., 2001). Low-grade mosaicism is likely to be missed. However, after karyotyping, FISH analysis of uncultured interphase amniocytes and chorionic villus cells may in fact aid diagnosis of any suspected constitutional mosaicism (Feldman *et al*., 2000; Siffroi *et al*., 2000). The FISH approach, which allows a higher number of interphase nuclei to be examined, may be of special advantage with respect to the problem of confined placental mosaicism in chorionic villus samples (Quilter *et al*., 2001).

QF–PCR

Mosaicism poses a challenge also when using QF–PCR for prenatal diagnosis. Mann *et al*. (2001) estimated that the QF–PCR assay is capable of identifying

autosomal mosaicism, where the trisomy is present in more than 10% of *in vitro* cultured cells. Cirigliano *et al*. (1999) were able to detect six out of seven cases of Turner syndrome with 46,XX; 45,X mosaicism of variable degrees, as indicated by karyotyping. In the single case, which was not detected by QF–PCR, the abnormal cells (45,X) were present in only 10% of cells of *in vitro* cultured amniocytes.

It may be added that prenatal diagnosis of constitutional mosacism is a very difficult problem altogether, not least because of the uncertain implications of mosaicism with respect to fetal and childhood development (Robinson *et al*., 2002). There is not a direct correspondence between the degree of mosaicism detected in different tissue samples, such as uncultured and cultured amniotic fluid and CVSs. Further research is urgently required to evaluate the clinical significance of different degrees of mosaicism, as detected in these different types of prenatal samples.

A trend towards 'stand-alone' molecular tests

Special attention has been paid to the potential for using FISH or QF–PCR for the diagnosis of common aneuploidies as 'stand-alone' tests. Opinions are divided. Bearing in mind that both amniocentesis and CVS are invasive procedures with a risk for induced abortion, it has been argued that follow-up by karyotyping should be performed to detect those chromosome abnormalities (in particular those associated with more or less severe physical and mental handicap) not identifiable by the current molecular tests. The proportion of such abnormalities, undetectable by current FISH and QF–PCR aneuploidy assays is about 20–30%, but varies considerably depending on the reasons for testing (Ward *et al*., 1993; Evans *et al*., 1999; Lewin *et al*., 2000; Pergament *et al*., 2000; Thein *et al*., 2000; Homer *et al*., 2003). For most women, when the indication for prenatal diagnosis is maternal age (in isolation or combined with maternal serum and ultrasonographic screening for fetal nuchal translucency), this risk is usually relatively low, in the order of 0.1–0.2% (Grimshaw *et al*., 2003). In other words, in the low-risk group of women, the abnormality detection rate is around 99.8–99.9%. In contrast, once a structural abnormality of the fetus has been diagnosed using ultrasonography, the risk may be substantially increased. The risk for a fetal chromosome abnormality is also substantially increased when either parent is a carrier of a chromosome rearrangement such as a translocation, inversion or insertion. Neither FISH nor QF–PCR aneuploidy assays are applicable. Either karyotyping or specific molecular testing (Chen *et al*., 2001) is mandatory.

Standard karyotyping also discovers structural chromosome rearrangements in balanced form, undetectable by current FISH and QF–PCR approaches. Carriers of structural chromosome rearrangements (such as translocations, inversions and insertions) are common in the general population and occur with an incidence of around one in 300. The incidence is not increased in amniotic fluid samples or CVSs, and the fetal carrier status may thus be a coincidental finding. Carrier status often runs in families. Detecting a structural chromosome rearrangement by prenatal diagnosis makes possible the unravelling of large families, where many carriers may be offered appropriate counselling regarding their reproductive risk (including reduced fertility and increased risks for abortions and stillbirths and for having a live-born child with a chromosome abnormality). This potential is lost by application of the molecular tests as a 'stand-alone' approach.

Summary and final remarks

FISH and QF–PCR constitute molecular methods that allow rapid and simple yet reliable prenatal diagnosis of targeted fetal chromosome disorders. Currently, these molecular tests are used for the detection of the most common abnormalities, trisomy 21, 13 and 18 (giving rise to the Down, Patau and Edward syndromes, respectively) and sex chromosome abnormalities (associated with the XXY–Klinefelter, XYY, XXX and X–Turner syndromes). These methods are applied to fetal non-dividing cells and DNA obtained using the invasive procedures amniocentesis and CVS. The methods are used mainly as a preamble to traditional karyotyping, performed after *in vitro* cell culture, using an aliquot of the same sample. However, a debate continues about the potential advantage for introducing these as 'stand-alone' tests for routine prenatal chromosome diagnosis in low-risk pregnancies (Ogilvie, 2003). This would be a highly cost-effective policy in relation to the current practice of both molecular and traditional testing (Grimshaw *et al*., 2003).

It is hoped that, in not too distant a future, the same technology may be applied for 'non-invasive' prenatal diagnosis on fetal cells or DNA retrieved from maternal blood samples, leading to a reduced requirement for invasive procedures that carry a risk for associated fetal loss. In the interim, the introduction of these targeted molecular methods *per se* may focus attention on the urgent need for appropriate information to be given to pregnant women (and their partners) regarding what fetal conditions may be looked for; and following discussions on the implications of the various disorders for child development, informed parental choices may be improved over and above the current situation (Marteau and Dormandy, 2001).

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Table 2. Small (short) tandem repeats (STRs) and primers commonly used for the detection of trisomies 21, 18, 13 and the sex chromosome abnormalities

Marker name	Heterozygosity (%)	Size of PCR product (bp)	Primer sequence	Chromosome location	Source and reference
D21S11	90.0	225–280	TATGTGAGTCAATTCCCCAAGTGA (F) GTTGTATTAGTCAATGTTCTCCAG (R)	21q21	GDB, Pertl *et al.*, 1996
D21S1411	93.0	⩾239	ATGATGAATGCATAGATGGATG (F) AATGTGTGTCCTTCCAGGC (R)	21q22.3	GDB, Pertl *et al.*, 1996
		256–340	GTAGATACATACATATGATGAATGC (F) TATTAATGTGTGTCCTTCCAGGC (R)		*Mann *et al.*, 2001
D21S1412	85.4	384–414	CGGAGGTTGCAGTGAGTTG (F) GGGAAGGCTATGGAGGAGA (R)	21q22.2	GDB, Pertl *et al.*, 1997
D21S1413	87.5	⩾180	TTGCAGGGAAACCACAGTT (F) TCCTTGGAATAAATTCCCGG (R)	21	GDB, Schmidt *et al.*, 2000
D21S1414 (amplifies the same region as D21S11 but produces a 122bp larger amplicon)	88.0	⩾291	GGCACCCAGTAAAAAATTACT (F) CTGTCTGTCTGTCTGTCTATC (R)	21q21	GDB
		330–380	AAATTAGTGTCTGGCACCCAGTA (F) CAATTCCCCAAGTGAATTGCCTTC (R)	21q21	*Pertl *et al.*, 1996
D21S1435	79.0	163–187	CCCTCTCAATTGTTTGTCTACC (F) GCAAGAGATTTCAGTGCCAT (R)	21q21	GDB, Cirigliano *et al.*, 2001
D21S1270	86.0	285–340	CTATCCCACTGTATTATTCAGGGC (F) TGAGTCTCCAGGTTGCAGGTGACA (R)	21q21–q22.1	Mann *et al.*, 2001
D21S226	59.0	440–470	GCAAATTTGTGGATGGGATTAACAG (F) AAGCTAAATGTCTGTAGTTATTCT (R)	21q22.1	Mann *et al.*, 2001
D21S1246	75.0	⩾400	GATAAAGTAGACAGGTAAACA (F) GGATTATAATTCAAGATGAGAT (R)	21q22.2	GDB
IFNAR (D21S2039)	83.0	231–251	TTACGTTCTTCATTTGATCTTAGCC (F) CCAGGCATGATGGCACAC (R)	21q22.1	GDB, Verma *et al.*, 1998
		450–500	GTTCTTCATTTGATCTTAGCCATC (F) GTGAGATAACTGGCAAGAAGATAA (R)	21q22.1	*Mann *et al.*, 2001
D13S631	83.0	189–223	GGCAACAAGAGCAAAACTCT (F) TAGCCCTCACCATGATTGG (R)	13q31–32	GDB, Pertl *et al.*, 1997
D13S634	81.2	⩾375	TCCAGATAGGCAGATTCAAT (F) CCTTCTTCTTCCCATTGATA (R)	13q14.3	GDB, Pertl *et al.*, 1997
		385–440	GGCAGATTCAATAGGATAAATAGA (F) GTAACCCCTCAGGTTCTCAAGTCT (R)	13q14.3	*Mann *et al.*, 2001
D13S258	83.3	230–267	ACCTGCCAAATTTTACCAGG (F) GACAGAGAGAGGGAATAAACC (R)	13q21.2–q31	GDB, Pertl *et al.*, 1999
D13S303	90.9	338–354	ACATCGCTCCTTACCCCATC (F) TGTACCCATTAACCATCCCCA (R)	13q22–q31	GDB, Schmidt *et al.*, 2000
D13S256	90.0	⩾154	CCTGGGCAACAAGAGCAAA (F) AGCAGAGAGACATAATTGTG (R)	13q14.1–q22	GDB, Schmidt *et al.*, 2000
D13S628	68.8	⩾247	ACGCCACTTTTCTAAATGCC (F) GGAGTAACAAATAGCAAGGCT (R)	13q31–q32	GDB

Continued.

Table 2. (*Continued*)

Marker name	Heterozygosity (%)	Size of PCR product (bp)	Primer sequence	Chromosome location	Source and reference
		425–470	TAACATTCATTGTCCCTTACAGAT (F) GCAAGGCTATCTAACGATAATTCA (R)	13q31–q32	*Mann *et al.*, 2001
D13S742	75.0	⩾364	TCCAGCCTGGTCAACACAG (F) TCCAGACTTCCCAATTCAGG (R)	13q11–q21.1	GDB
		235–315	ATAACTGGGCTAGGAATGGAAATA (F) GACTTCCCAATTCAGGAGGACT (R)	13q11–q21.1	*Mann *et al.*, 2001
D13S305	75.0	430–465	GCCTGTTTGAGGACCTGTCGTTA (F) TGGTTATAGAGCAGTTAAGGCAC (R)	13q12.1–q14.1	GDB, Mann *et al.*, 2001
MBP	LOCUS A:80 LOCUS B:79	A: 122–124 B:208–232	GGACCTCTGGAATTACAATC (F) ATTTAACCTACCTGTTCATCC (R)	18q23–ter	GDB, Pertl *et al.*, 1996
D18S51	88.5	271–331	GAGCCATGTTCATGCCACTG (F) CAAACCCGACTACCAGCAAC (R)	18q21.33	GDB
	80.2	279–323	CCAACCCGACTACCAGCAAC (F) GAGCCATGTTCATGCCACTG (R)	18q21.33	*Schmidt *et al.*, 2000
D18S499	71.0	150–178	CTGCACAACATAGTGAGACCTG (F) AGATTACCCAGAAATGAGATCAGC (R)	18q21.32–q21.33	GDB, Schmidt *et al.*, 2000
D18S380	66.7	⩾151	CACTGCATTCTGGGCAAC (F) AGGCTCTTGCTCCTGGAAT (R)	18q22.3–q23	GDB
		160–200	GCATTCTGGGCAACAAGGTGAAAC (F) GAGATAACCCAGGCAAGAACAGGA (R)	18q22.3–q23	*Mann *et al.*, 2001
D18S391	75.0	⩾182	CTGGTTTTCGTCTTGAGAAG (F) CACTATTCCCATCTGAGTCA (R)	18pter–18p11.22	GDB
		140–180	GGACTTACCACAGGCAATGTGACT (F) TAGACTTCACTATTCCCATCTGAG (R)	18pter–18p11.22	*Mann *et al.*, 2001
D18S535	74.6	126–156	TCATGTGACAAAAGCCACAC (F) AGACAGAAATATAGATGAGAATGCA (R)	18q12.2	GDB, Pertl *et al.*, 1999
	92.0	455–500	CAGCAAACTTCATGTGACAAAAGC (F) CAATGGTAACCTACTATTTACGTC (R)	18q12.2–q12.3	*Mann *et al.*, 2001
D18S1002	81.2	286–318	CAAAGAGTGAATGCTGTACAAACAGC (F) CAAGATGTGAGTGTGCTTTTCAGGAG (R)	18q11	GDB, Schmidt *et al.*, 2000
D18S386	92.3	330–387	TCAGGAGAATCACTTGGAAC (F) TCCATGAAGTAGCTAAGCAG (R)	18q22.1–q22.2	GDB, Pertl *et al.*, 1999
	87.5	330–400	TGAGTCAGGAGAATCACTTGGAAC (F) CTCTTCCATGAAGTAGCTAAGCAG (R)	18q22.1–q22.2	*Mann *et al.*, 2001
D18S858	82.0	193–211	AGCTGGAGAGGGATAGCATT (F) TGCATTGCATGAAAGTAGGA (R)	18	GDB, G. Voglino personal communication
XHPRT	73.3	260–302	ATGCCACAGATAATACACATCCCC (F) CTCTCCAGAATAGTTAGATGTAGGTAT (R)	Xq26.1	Pertl *et al.*, 1997
DXS8377	95.0	203–245	CACTTCATGGCTTACCACAG (F) GACCTTTGGAAAGCTAGTGT (R)	Xq26	GDB, Schmidt *et al.*, 2000

DXS6803	86.7	106–125	GAAATGTGCTTTGACAGGAA (F) CAAAAAGGGACATATGCTACTT (R)	Xpter–qter	GDB, Cirigliano *et al.*, 2001
DXS6809	86.7	242–274	TGAACCTTCCTAGCTCAGGA (F) TCTGGAGAATCCAATTTTGC (R)	Xpter–qter	GDB, Cirigliano *et al.*, 2001
X22	85.2	189–242	TAATGAGAGTTGGAAAGAAA (F) CCCATTGTTGCTACTTGAGA (R)	Xq28 (PAR2)	Cirigliano *et al.*, 1999
AMXY		X–432 Y–250	CTGATGGTTGGCCTCAAGCCT (F) ATGAGGAAACCAGGGTTCCA (R)	X and Y	Pertl *et al.*, 1996
		X–106 Y112	CCCTGGGCTCTGTAAAGAATAGT (F) ATCAGAGCTTAAACTGGGAAGCTG (R)	Xp22.1–22.31 Yp26.1	Cirigliano *et al.*, 1999
SBMA	90.0	142–178	TCC GCG AAG TGA AGA AC (F) CTT GGG GAG AAC CAT CCT CA (R)	Xq11.2–q12	Schmidt *et al.*, 2000
DXS981	86.0	230–260	CTCCTTGTGGCCTTCCTTAAATG (F) TTCTCTCCACTTTTCAGAGTCA (R)	Xq11.2–q13.1	GDB, Donaghue *et al.*, 2003
DXS6854	73.0	90–125	AGCACTTCTCCTACAACCCTC (F) CAGCCTGGGCAGTAGAGACT (R)	Xq26	GDB, Donaghue *et al.*, 2003
P39	87.0	140–166	AGCACATGGTATAATGAACCTCCACG (F) CAGTGTGAGTAGCATGCTAGCATTTG (R)	Xq28	Donaghue *et al.*, 2003
DXS996	82.0	130–168	AAATTCTTGCTTAGGCCACTCTAGG (F) AACGTTGTTCTGGATCGTATGCTA (R)	Xp22.3	GDB, Donaghue *et al.*, 2003
DXS337	83.0	163–193	TGCATCATTCAGCTTTCAGG (F) GTGACAGAGTGAGACCCTGTC (R)	Xp11.3–p11.23	GDB, Pertl *et al.*, 1999
DXS1283	95.0	203–245	AGTTTAGGAGATTATCAAGCTGG (F) GTTCCCATAATAGATGTATCCAG (R)		Schmidt *et al.*, 2000

*These primer sequences have been modified since their first use.
GDB: Genome database.
The references in this table are examples in which these particular markers have been used successfully in quantitative fluorescence polymerase chain reaction (QF–PCR) for the prenatal detection of chromosomal aneuploidies and do not refer to the groups who had first published the sequences.

Table 3. Examples of recent studies of prenatal diagnosis using quantitative fluorescence polymerase chain reaction (QF–PCR) with markers specific for chromosomes 21, 18, 13, X and Y

Number of cases analysed by QF–PCR/Total	Chromosomes tested	Abnormalities detected/total analysed by PCR (%)	Abnormalities not detected/total analysed by PCR (%)	Number of STR markers used	Informativeness (%)	Heavily blood stained samples (%)	Reference
2083/2167	21	32 (1.5)	0	3	99.6	1.3	Verma *et al.*, 1998
Total 2083		**32 (1.5)**	**0 (0)**				
662/662	21	5 (0.8)	0	3	98.3	1.0	Schmidt *et al.*, 2000
	18	4 (0.6)	1 (0.02)	3	97.3		
	13	0 (0.0)	0	3	94.6		
	XY	45,X–4 (0.6)	0	3			
		47,XXY–1 (0.2)					
Total 662		**14 (0.3)**	**1 (0.02)**				
5000/5097	21	57 (1.1)	0	6	98.0 overall	2.0	Levett *et al.*, 2001
	18	17 (0.3)	0	6			
	13	8 (0.2)	0	6			
	XY	16 in total (0.3)	4	X–5			
				Y–2			
Total 5000		**98 (1.9)**	**4 (0.1)**				
1314/1373	21	55 (4.2)	0	4	99.5 overall	2.0	Mann *et al.*, 2001
	18	23 (1.8)	0	4			
	13	10 ((0.8)	0	4			
Total 1314		**88 (6.7)**	**0 (0)**				
5090/5090	21	189 (3.7)	0	4 (2*)	99.5 overall	1.5	K. Mann, personal communication
	18	75 (1.5)	0	4 (2*)			
	13	32 (0.6)	0	4 (2*)			
Total 5090		**296 (5.8)**	**0 (0)**				
3478/3478	21	58 (1.7)	0		98.8	1.2	V. Cirigliano, personal communication, ISPD conference, 2002
	18	28 (0.8)	0		98.5		
	13	7 (0.2)	0				
	XY	14 in total (0.4)	0				
Total		**107 (3.1)**	**0 (0)**				
1653/1653	21	110 (6.6)	0		98.9	Not recorded	Voglino *et al.*, 2002
	18	40 (2.4)	0				
	13	15 (0.9)	0				
	XY	45,X–18 (1.1)	0				
		47,XXY–4 (0.2)	0				
		47,XYY–3 (0.2)	0				
Total 1653		**187 (11.3)**	**0 (0)**				
Grand total 17 966		**822 (4.6)**	**5 (0.03)**				

Note the relatively low proportion of abnormalities missed in these studies, in total five of 17 966 (0.03%). In addition, note that the informativeness (proportion of cases showing heterozygosity for the markers concerned) is usually in the order of 98–99%. Triploidy and cases of mosaicism are not included.
If all the primary markers are uninformative, additional ones may be used to increase the informativeness. Number of additional markers used is indicated by an *.
STR: small (short) tandem repeat.

Table 4. Examples of large or recent prenatal diagnosis studies using fluorescence *in situ* hybridization (FISH) probes specific for chromosomes 21, 18, 13, X and Y

Number of cases analysed by FISH/total	Chromosomes tested	Abnormalities detected/total analysed by FISH (%)	Abnormalities not detected/total analysed by FISH (%)	Probes used	Informativeness (%)	Blood stained samples (%)	Reference
4059/4500	21	38 (0.9)	5 (0.1) false neg	Cosmids	99.2	3.8	Ward *et al.*, 1993
	18	30 (0.7)	1 (0.02) false neg				
	13	12 (0.3)	1 (0.02) false neg				
	XY	45,X–12 (0.3)	1 (0.02) false pos				
		47,XXY–4 (0.1)					
		47,XXX–4 (0.1)					
Total 4059		**100 (2.5)**	**8 (0.2)**				
2523/2709	21	48 (0.02)	0	Cosmids	93.0	0	Bryndorf *et al.*, 1996
	18	14 (0.01)	0				
	13	10 (0.003)	0				
	XY	8 in total (0.003)	0				
Total 2523		**80 (0.03)**	**0**				
2079/2154	21	48 (2.3)	0	Yac 831B9	99.7	2.9	Morris *et al.*, 1999
Total 2079		**48 (2.3)**	**0**				
2336/2336	21	24 (0.9) in total	0	Aneuvysion, Vysis	100.0	Not recorded	Pergament *et al.*, 2000
	18						
	13						
	XY						
Total 2336		**24 (0.9)**	**0**				
3202/3202	21	45 (1.4)	0	Aneuvysion, Vysis	99.9	Not recorded	Thilaganathan *et al.*, 2000
	18	25 (0.7)					
	13	5 (0.2)					
	XY	45,X–4 (0.1)					
		47,XXY–4 (0.1)					
Total 3202		**83 (2.6)**	**0**				
301	21	14 (4.7)	0	Oncor and Aneuvysion, Vysis	100.0	Not recorded	Feldman *et al.*, 2000
	13	3 (0.9)	0				
	18	10 (3.3)	0				
	XY	45,X–4 (1.3)	0				
Total 301		**31 (9.9)**	**0**				
309/309	21	21 (6.8)	1 (0.3) false neg	Oncor and Aneuvysion, Vysis	95.1 (7/15 late 25–40 week amnios)	Not recorded	Cheong Leung *et al.*, 2001
	18	12 (3.8)	1 (0.3) false neg				
	13	3 (1.0)	2 (0.6) false neg				
	XY	45,X–4 (1.3)	0				
		47,XXY–2 (0.6)	0				
Total 309		**42 (13.6)**	**4 (1.3)**				

Continued.

Table 4. (*Continued*)

Number of cases analysed by FISH/total	Chromosomes tested	Abnormalities detected/total analysed by FISH (%)	Abnormalities not detected/total analysed by FISH (%)	Probes used	Informativeness (%)	Blood stained samples (%)	Reference
5197/5348	21	183 (3.5)	0	Aneuvysion, Vysis	97.2	0.2	Tepperberg *et al.*, 2001
	18	195 (3.8)	1 (0.02)				
	13	73 (1.4)	1 (0.02)				
	XY	45,X–64 (1.2)	0				
Total 5197		**515 (9.9)**	**0**				
911/11123	21	35 (3.8)	3 false neg	Oncor and Aneuvysion, Vysis	97.0	Not recorded	Weremowicz *et al.*, 2001
	18	21(2.3)	2 false neg				
	13	5 (0.5)	0				
	XY	45,X–6 (0.6)	0				
		47,XXY–1 (0.1)	0				
Total 911		**68 (7.5)**	**5 (0.5)**				
5049/5049 for chromosome 21, not recorded for 18, 13, X and Y	21	70 (1.4)	0	Aneuvysion, Vysis	99.7	1.3	Witters *et al.*, 2002
	18	15 (0.3)	0				
	13	5 (0.1)	0				
	XY	45,X–12 (0.2)	0				
		47,XXY–1 (0.02)					
		47,XXX–1 (0.02)					
Total 5049		**104 (2.1)**	**0**				
Grand total 20 453		**967 (4.7)**	**17 (0.08)**				

Note the relatively low proportion of abnormalities missed in studies during the last three years, in total nine of 14 187 (0.06%). In addition, note that the informativeness (proportion of cases where a diagnosis could be obtained) is usually over 99%. Triploidy and cases of mosaicism are not included.

Table 5. Type and reason for misdiagnosis by fluorescence *in situ* hybridization (FISH) using the different types of probes

Probe used	Source of probe	FISH signals	Case karyotype	Potential misdiagnosis	Explanation given	Reference
13/21	Not specified	+13/21	46,XX or XY	False positive trisomy 21/trisomy 13 or false negative normal if trisomy 21/trisomy13	Probe hybridized to chromosome 22	Strovel *et al.*, 1992
D13Z1/ D21Z1	Oncor	+13/21	46,XX	False positive trisomy 21/trisomy 13 or false negative normal if trisomy 21/trisomy 13	Probe hybridized to chromosome 22	Verlinsky *et al.*, 1995
L1.26	Not specified	+13/21	46,XY	False positive trisomy 21/trisomy 13 or false negative normal if trisomy 21/trisomy13	Probe hybridized to chromosome 22	Tardy and Toth, 1997
L1.26	Devilee, 1986	Two extra 13/21 signals	47, +21	False positive tetrasomy 21/tetrasomy 13	Probe hybridized to chromosome 14	Lapidot–Lifson *et al.*, 1996
D13Z1/ D21Z1	Not specified	–13/21	46,XX	False positive monosomy 21/13 or false negative normal if trisomy 21/13	Pericentromeric deletion of chromosome 21, maternally inherited	Mizunoe and Young, 1992
13/21 PCR fragment	Homebrew	–13/21	46,XX or XY	False positive monosomy 21/13 or false negative normal if trisomy 21/13	Weak chromosome 21 signal due to centromeric deletion	Weier and Gray, 1992
Not specified	Pinkel, 1986	–13/21	46,XY	False positive monosomy 21/13 or false negative normal if trisomy 21/13	Heteromorphism in centromeric area of chromosome 21	Seres–Santamaria *et al.*, 1993
D13Z1/ D21Z1	Devilee, 1986	–13/21	47, +21 two cases	False negative normal or false positive monosomy 21/13 if normal	Deletion in centromeric sequences, paternally inherited	Verma and Luke, 1992
D13Z1/ D21Z1	Oncor	–13/21	47, +21 two cases	False negative normal or false positive monosomy 21/13 if normal	Variations in signal intensity not allowing differentiation between normal and abnormal signals	Cacheux *et al.*, 1994
D13Z1/ D21Z1	Oncor	–13/21	47, +13 two cases	False negative normal or false positive monosomy 21/13 if normal	Variations in signal intensity not allowing differentiation between normal and abnormal signals	Cacheux *et al.*, 1994
D13Z1/ D21Z1	Oncor	–13/21	47, +21 three cases	False negative normal or false positive monosomy 21/13 if normal	Polymorphism or hybridization insufficiency?	Verlinsky *et al.*, 1995
Cosmid contig 21q22.3	Klinger *et al.*, 1992	–21	47, +21 two cases	False negative normal or false positive monosomy 21 if normal	High background fluorescence and autofluorescence of microscope objective	Ward *et al.*, 1993

Continued.

Table 5. (*Continued*)

Probe used	Source of probe	FISH signals	Case karyotype	Potential misdiagnosis	Explanation given	Reference
Cosmid contig 21q22.3	Klinger *et al.*, 1992	−21	47, +21 three cases	False negative normal or false positive monosomy 21 if normal	Maternal cell contamination	Ward *et al.*, 1993
Not specified	Pinkel, 1986	−21	47, +21 three cases	False negative normal or false positive monosomy 21 if normal	Pericentromeric deletion	Seres–Santamaria *et al.*, 1993
L1.26	Devilee, 1986	−21	47, +21 three cases	False negative normal or false positive monosomy 21 if normal	Pericentromeric deletion	Bossuyt *et al.*, 1995
L1.26	Devilee, 1986	−21	47, +21	False negative normal or false positive monosomy 21 if normal	Maternal cell contamination	Bryndorf *et al.*, 1997
LSI 21q22.13–21q22.2	Vysis	−21	47, +21	False negative normal or false positive monosomy 21 if normal	Maternal cell contamination	Estabrooks *et al.*, 1999
LSI 21q22.13–21q22.2	Vysis	+21	46,XX	False positive trisomy 21/trisomy 13 or false negative normal if trisomy 21/trisomy 13	Unexplained (not cryptic rearrangement or vanishing twin)	Weremowicz *et al.*, 2001
LSI 21q22.13–21q22.2	Vysis	+21	46,XY	False positive trisomy 21/trisomy 13 or false negative normal if trisomy 21/trisomy13	Unexplained (not cryptic rearrangement or vanishing twin)	George *et al.*, 2003
Cosmid contig 13q12.3	Klinger *et al.*, 1992	−13	47, +13	False negative normal or false positive monosomy 13 if normal	Unexplained	Ward *et al.*, 1993
LSI 13q14	Vysis	−13	47, +13	False negative normal or false positive monosomy 13 if normal	Maternal cell contamination, poor hybridization	Estabrooks *et al.*, 1999
LSI 13q14	Vysis	−13	47, +13	False negative normal or false positive monosomy 13 if normal	Large deletion at RBI locus?	Tepperberg *et al.*, 2001
D18Z1	Vysis	+18	46,XX or XY	False positive trisomy 18 or false negative normal if trisomy	Cross hybridization to chromosome 22	Thangavelu *et al.*, 1998
18q23	Klinger *et al.*, 1992	−18	47, +18	False negative normal or false positive monosomy if normal	High background fluorescence and autofluorescence of microscope objective	Ward *et al.*, 1993
D18Z1	Vysis	−18	47, +18	False negative normal or false positive monosomy if normal	Maternal cell contamination	Estabrooks *et al.*, 1999
D18Z1	Vysis	−18	47, +18	False negative normal or false positive monosomy if normal	Weak signal from reduced copy number of alpha satellite sequences	Thilaganathan *et al.*, 2000

D18Z1	Vysis	−18	47, +18	False negative normal or false positive monosomy if normal	Weak signal from reduced copy number of alpha satellite sequences	Tepperberg *et al.*, 2001
DXZ1	Oncor	+X	46,XX,t(15;Y) (p11.2;q11.21)	False positive XXX	Constitutionally abnormal X chromosome	Verlinsky *et al.*, 1998
DXZ1	Oncor	+X	46,XY	False positive XXY	Cross hybridization to chromosome 19	Winsor *et al.*, 1999
DXZ1	Vysis	+X	45,X	False negative normal or false positive XXX if normal	Extraembryonic cell contamination in specimen? Not confirmed by postmortem	Eiben *et al.*, 1998
Centromeric X fragment	Klinger *et al.*, 1992	−X	46,XX	False positive 45,X or false negative normal if 47,XXX	Weak X signal	Bryndorf *et al.*, 1996
DXZ1	Vysis	−X	46,XX	False positive 45,X or false negative normal if 47,XXX	Weak X signal	Tsuchiya *et al.*, 2001
DYZ1	Oncor	+Y	46,XY,t(15;Y) (p11.2;q11.21)	False positive 47,XYY	Constitutionally abnormal Y chromosome	Verlinsky *et al.*, 1998
PDP97 derivative	Oncor	−Y	46,XY	False positive 45,X	Failure of Y probe to hybridize	Ward *et al.*, 1993
PHY2.1	Pr. Cooke	−Y	46,X,der(Yp)	False positive 45,X	Abnormal Y chromosome	Mercier and Bresson, 1995
PDP97	Wolfe, 1985	−Y	47,XXY	False negative	Maternal cell contamination, failure of Y probe to hybridize	Bryndorf *et al.*, 1997
DYZ3	Vysis	−Y	46,XY	False negative	Weak Y signal, from reduced copy number of alpha satellite sequences	Tepperberg *et al.*, 2001

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