

Rapid Aneuploidy Testing, Traditional Karyotyping, or Both, in Prenatal Diagnosis

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The accuracy of new molecular diagnostics, fluorescence in-situ hybridisation (FISH) or quantitative fluorescence-polymerase chain reaction (QF-PCR), collectively known as rapid aneuploidy testing (RAT), in prenatal diagnosis has already been demonstrated in a number of large studies. The challenge now is how to apply them clinically in the most cost-effective manner. There is currently a hot debate in this area of prenatal diagnosis.

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Introduction

The most frequent foetal chromosomal abnormalities involve the autosomes 21, 18, 13 and sex chromosomes X and Y. Aneuploidy or alterations in copy number of these chromosomes, including Trisomy 21 (Down syndrome), Trisomy 18 (Edwards syndrome), Trisomy 13 (Patau syndrome), 45,X (Turner syndrome), 47,XXY (Klinefelter syndrome) and Triploidy (presence of 3 copies of each chromosome), account for more than 80% of clinically significant chromosomal abnormalities diagnosed in the prenatal period. Down syndrome is a well-recognised cause of mental retardation, cardiac and other congenital abnormalities. Edwards syndrome and Patau syndrome lead to multiple congenital abnormalities and early neonatal death. The phenotype of Turner syndrome is highly variable including short stature, amenorrhoea, infertility, cardiac and renal malformations. Klinefelter syndrome is associated with a relatively mild phenotype. Foetuses with triploidy are severely growth retarded and usually die in-utero.

The traditional standard method for prenatal diagnosis of these common aneuploidies involves

analysis of banded metaphase chromosomes from cultured amniotic fluid cells (amniocentesis) or chorionic villi (chorionic villous sampling). It is known as karyotyping. Apart from the common aneuploidies, all the 23 pairs of chromosomes are examined. A wide range of chromosomal abnormalities can be identified, including aneuploidy as well as chromosomal rearrangements, such as translocations and inversions that may be balanced or unbalanced. Traditional karyotyping is labour intensive and results are not usually available for 2 weeks or more. Advances in molecular diagnostics, using either fluorescence in situ hybridisation (FISH) with chromosome specific DNA probes or quantitative fluorescence-polymerase chain reaction (QF-PCR) with chromosome specific small tandem repeat markers, can be applied to diagnose these common aneuploidies within 1 to 2 days. The sensitivity and specificity of FISH

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and QF-PCR, collectively described as rapid aneuploidy testing (RAT), have been demonstrated in a number of large-scale studies. They compare favourably with traditional karyotyping for the diagnosis of the common aneuploidies. Unlike karyotyping, these technologies only allow the identification of the chromosomal abnormalities that are specifically sought.

If RAT (FISH or QF-PCR) is used to give a preliminary rapid result for the common aneuploidies as an adjunct to karyotyping, it will increase the cost of prenatal diagnosis. Some suggest that if the indication for prenatal diagnosis is an increased risk of Down syndrome, such as positive screening test result or advanced maternal age, karyotyping should be effectively replaced by RAT. Nonetheless, this new approach is not supported by others because certain chromosomal abnormalities, although of a small number and might not be clinically significant, would be missed¹.

Fluorescence In Situ Hybridisation

FISH²⁻⁶ involves hybridisation 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 visualised under the microscope (Figure 1). Normal samples show 2 dots per cell nucleus, whereas trisomic samples show 3 dots. 50 to 100 cells are usually analysed to allow for low-level background and signal overlay that occur during FISH procedures.

Quantitative Fluorescence-Polymerase Chain Reaction

QF-PCR⁷⁻¹² involves the amplification of chromosome-specific repeated DNA sequences known as small tandem repeats (STRs). STRs are stable and polymorphic, varying in length between subjects, depending on the number of times the tri-, tetra- or penta-nucleotides are repeated. The sample DNA from amniotic fluid or chorionic villi is amplified by PCR using fluorescent primers so that products can be visualised and quantified as peak areas of the respective repeat lengths using an automated DNA sequencer with the gene-scan software (Figure 2). DNA amplified from normal subjects who are heterozygous (having alleles of different STR lengths) will show 2 peaks with the same area. DNA amplified from trisomic subjects will show either an extra peak (triallelic) with the same area, or only 2 peaks (diallelic), one of them twice as large as the other. The number and variety of STR markers multiplexed together differ between assays and determine assay efficiency.

Other Molecular Methods for RAT

Other PCR-based approaches to RAT¹³ include homologous gene quantitative PCR (HGQ-PCR)¹⁴ and real-time PCR¹⁵. In addition, multiplex ligation-dependent probe amplification (MLPA)¹⁶ and microarray comparative genomic hybridisation (CGH)¹⁷ can also be used for RAT. At present, these other methods are less extensively studied when compared to FISH or QF-PCR in RAT and discussion of their details is beyond the scope of this article.

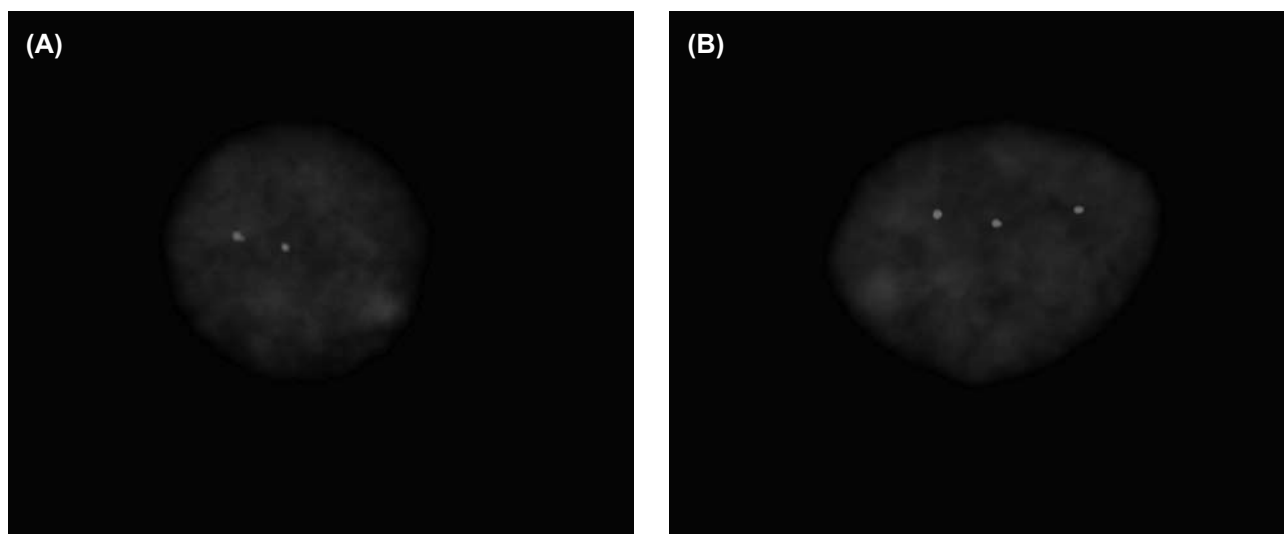


Figure 1. FISH assays on interphase nuclei of uncultured amniotic fluid cells probed with LSI 21 (Vysis) for 21q22.13 to 21q22.2 region on the long arm of chromosome 21. Two signals detected from a normal sample (A) and three signals detected from a sample with Trisomy 21 (B)

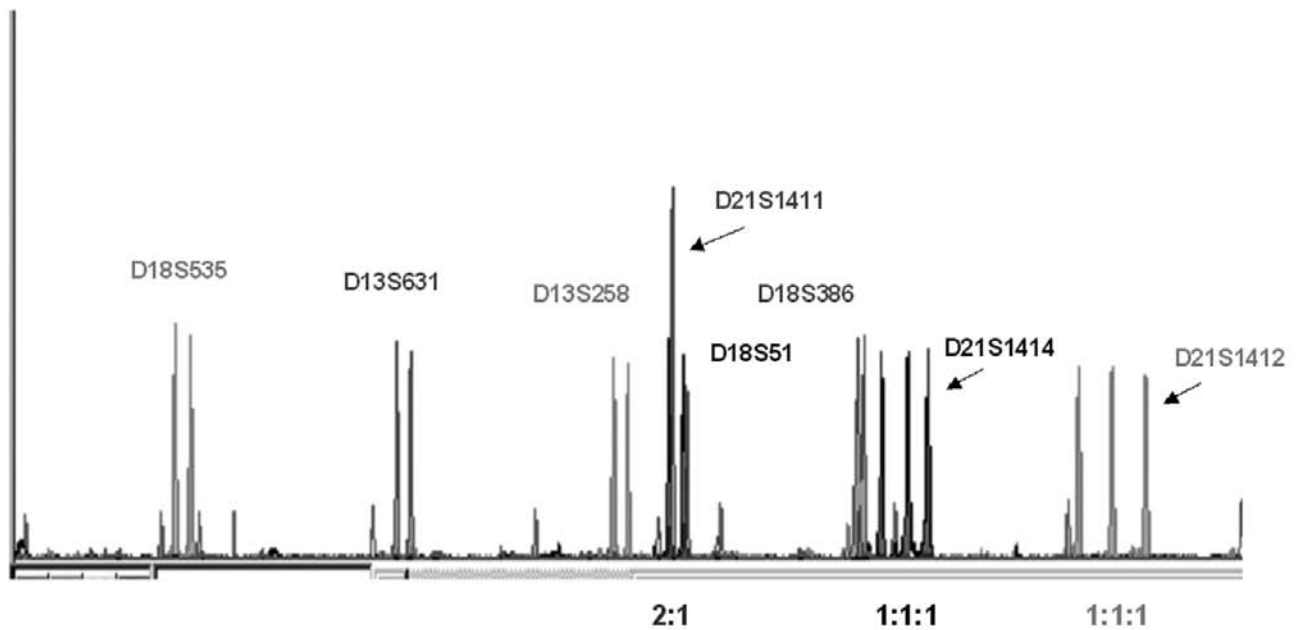


Figure 2. QF-PCR with STR markers showing Trisomy 21 from amniotic fluid. Arrows showed diallelic pattern with ratio 2:1 (D21S1411) or triallelic patterns with ratio 1:1:1 (D21S1414, D21S1412). Normal patterns (1:1 ratio) are observed for chromosome 18 (D18S535, D18S51, D18S386) and chromosome 13 (D13S631, D13S258)

QF-PCR Versus FISH

Comparing QF-PCR with FISH¹⁸:

1. The risk for misdiagnosis of the common aneuploidies by either FISH or QF-PCR is relatively small.
2. FISH is more labour intensive than QF-PCR.
3. Maternal cell contamination may constitute more of a problem with FISH than with QF-PCR.
4. Foetal mosaicism remains a challenge by either method.

Maternal Cell Contamination

Maternal cell contamination of foetal material may arise during any of the invasive prenatal sampling procedures. With FISH, mixture of maternal and foetal XY cells are readily detectable but maternal and foetal XX cells are indistinguishable. Using QF-PCR, maternal cell contamination is readily detected by the characteristic pattern with extra alleles or skewed ratios between peaks for all target chromosomes¹⁹.

Foetal Mosaicism

It refers to the occurrence of more than one cell line containing different chromosomal results. Using FISH, examination of a large number of interphase nuclei facilitates the diagnosis of mosaicism. Low-

grade mosaicism is likely to be missed. QF-PCR is also capable of identifying autosomal mosaicism, when the trisomy is present in more than 15%²⁰.

RAT Versus Karyotyping

When the results of RAT and karyotyping are compared, they can be divided into 4 groups: concordant normal, concordant abnormal, false positive and false negative. Table 1 shows a comparison of RAT and karyotyping results from 12 studies from 1999 to 2004^{6,12,21-32}.

Concordant Normal

Concordant normal RAT with normal karyotyping results is the most common scenario (96.7%). In other words, karyotyping does not give additional information to RAT in the great majority of women having prenatal tests such as amniocentesis or chorionic villous sampling. Most of these prenatal tests are performed because of positive Down screening test result or advanced maternal age. The major advantage of RAT in these women is that the rapid normal result within 1 to 2 days can relieve the anxiety of the women and their partners much earlier than when they have to wait for the karyotyping result which can take up to 3 weeks. However, a randomised controlled trial has suggested that this advantage of

Table 1. Comparison of rapid aneuploidy testing (RAT) and karyotyping results^{6,12,21-32}

| | Normal karyotype | Abnormal karyotype | Total |
|--------------|------------------|--------------------|-----------------|
| Normal RAT | 225,817 (96.7%) | 2,039 (0.9%) | 227,856 (97.6%) |
| Abnormal RAT | 1 (0.0%) | 5,639 (2.4%) | 5,640 (2.4%) |
| Total | 225,818 (96.7%) | 7,678 (3.3%) | 233,496 (100%) |

RAT may be lost if the women still have to wait for the karyotyping result³³. One possible explanation is that the woman, although being told that the RAT result is normal, is still having a significant degree of anxiety as she has to wait for the 'confirmation' by the karyotyping result. This anxiety can be alleviated if the RAT report is considered to be final.

Concordant Abnormal

Concordant abnormal RAT with the same abnormal karyotyping results accounts for 2.4% of the results (Table 1). In this group of women, karyotyping does not give additional information to RAT. At present, many centres³⁴, including ours, offer the option of termination of pregnancy based on abnormal RAT results without karyotyping confirmation. In this regard, RAT is again considered as a stand-alone test.

False Positive

This refers to the scenario when the RAT result is falsely abnormal in the presence of a normal karyotype which can potentially result in termination of a normal pregnancy. It has occurred in 1 out of the 233,496 cases in the 12 recent studies that have been reviewed (Table 1)²². This case was a 45,X / 46,XX mosaic predicted by FISH but 46,XX found on karyotyping. The author explained that it was the result of extreme variation in size of the alpha satellite centromeric region of the X chromosome. This was a rare occurrence and manifested as very low signal strengths on fluorescent microscopy. When very low signal strengths are encountered, FISH analysis is repeated with alternative probes or reliance should be placed on karyotyping. Nevertheless, the absence of false-positive result is a basic prerequisite if RAT is to be used as a stand-alone test.

False Negative

This refers to the scenario when the RAT result is normal but the karyotyping result is abnormal. It accounts for 0.9% of the results (Table 1). These 'abnormal'

karyotypes are divided into 2 groups: clinically not significant (0.6%) and clinically significant (0.4%). The clinically not significant group refers to those balanced translocations or other chromosomal rearrangements of known familial origin. The clinically significant group includes rare aneuploidies (other than chromosomes 21, 18, 13, X and Y), unbalanced translocations or other chromosomal rearrangements, balanced de novo translocations and marker chromosomes. Strictly speaking, they should not be considered as false-negative RAT results because FISH or QF-PCR can only detect the common aneuploidies (chromosomes 21, 18, 13, X and Y). However, this is the group of chromosomal abnormalities that will be missed if RAT is to replace karyotyping.

Some, but not all, of the clinically significant chromosomal abnormalities that cannot be detected by RAT have evidence of major structural abnormalities or soft markers of aneuploidy on ultrasound examination^{23,28}. A policy offering RAT to all patients, but restricting karyotyping to cases with ultrasound anomalies, may reduce the number of karyotyping by 70%, but maintain a 95% detection rate for all clinically important chromosomal abnormalities³¹.

Nevertheless, there may be some clinically significant false-negative RAT chromosomal abnormalities that do not have ultrasound abnormalities. It is important to note that the clinical significance of these chromosomal abnormalities, in particular, balanced de novo translocations and marker chromosomes, is very different from that of Trisomy 21, 18 or 13³⁵. The risk of an adverse clinical outcome (including impaired intellectual development, learning difficulties and physical abnormalities) for this cohort of chromosomal abnormalities varies from 5 to 15%³⁵. Identification of these balanced de novo translocations and marker chromosomes in the absence of ultrasound abnormalities often poses difficult counselling issues, may not be in the best interest

of the parents or the foetus, and presents a difficult choice regarding the continuation of the pregnancy.

In addition to the above clinically significant chromosomal abnormalities, false-negative RAT results also include those balanced translocations or other chromosomal rearrangements of known familial origin. Although they are not clinically significant, they have the potential to result in unbalanced products in future pregnancies.

Cost-effectiveness

One of the advantages of using RAT as a stand-alone test is cost-saving. Instead of adding the cost of RAT on top of that of karyotyping, the cost of the latter can be saved by the RAT-alone approach. In the age of ever-escalating cost in the provision of health care, especially in a government-funded public medical care system, the savings can be redirected to enhance existing or fund new programmes, thus maximising the effect of limited resources. Grimshaw et al³⁰ has conducted a cost-effectiveness analysis on 5 testing policies:

1. RAT and karyotyping for all women
2. RAT as a replacement for karyotyping
3. RAT for all plus karyotyping for high-risk women
4. Karyotyping for all plus RAT for high-risk women
5. Parental choice plus karyotyping for high-risk women

Policies 2, 3 and 5 are found to be more cost-effective than karyotyping based on the cost per case (chromosomal abnormality) detected.

Ethical Issue

If RAT is to replace karyotyping for indications such as positive Down screening or advanced maternal age when no ultrasound abnormality is detected, one has to accept the risk that for every 1000 amniocenteses

performed, up to 4 potentially clinical significant chromosomal abnormalities may be missed. Some people may argue from an ethical point of view that since amniocentesis is an invasive procedure that carries a small risk of miscarriage, we should maximise the information that can be obtained by performing karyotyping to examine all the 23 pairs of chromosomes. However, even the performance of karyotyping does not mean that the information is maximised e.g. microdeletions and common mutations are not tested. We must also realise that Down screening is a programme designed to detect primarily Down syndrome and therefore in principle, follow-up test with RAT alone would have realistically fulfilled the expectations of the couples and obstetricians (targeted testing). A recent conjoint analysis study showed that women would prefer simple information on just knowing whether the foetus has Down syndrome as long as the result is received 6 days sooner than karyotyping³⁶.

Conclusion

The major advantages of RAT (FISH or QF-PCR) include fast reporting within 24 to 48 hours and earlier relief of anxiety. Their accuracy in prenatal diagnosis has already been demonstrated. The challenge now is how to apply them clinically in the most cost-effective manner. Future research should focus on whether RAT could replace karyotyping when prenatal tests are performed for indications such as positive Down screening test or advanced maternal age. A high standard ultrasound examination is essential for this new approach. Using this new approach in prenatal diagnosis, for every 1000 amniocenteses performed, up to 4 potentially clinical significant chromosomal abnormalities may be missed e.g. balanced de novo translocations and the presence of marker chromosomes.

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