

# New Molecular Techniques for the Prenatal Detection of Chromosomal Aneuploidy

This technical update has been prepared by the Genetics Committee and approved by the Executive and Council of the Society of Obstetricians and Gynaecologists of Canada.

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techniques and the evidence supporting their use in prenatal diagnosis. These methods are reliable and cost-effective for detecting the targeted fetal aneuploidies, but are limited in their ability to detect non-aneuploid chromosome abnormalities, some of which are clinically significant.

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## Summary Statements

1. Currently available methods for rapid aneuploidy detection (RAD) include fluorescence in situ hybridization (FISH) and quantitative fluorescence polymerase chain reaction (QF-PCR). Multiplex ligation-dependent probe amplification (MLPA) is a newer technology under investigation.
2. FISH and QF-PCR are known to, and MLPA is proving to, have similar sensitivity and specificity to full cytogenetic karyotyping (the current "gold standard") for the detection of fetal aneuploidy (for chromosomes 13, 18, and 21 and the sex chromosomes). Advantages of QF-PCR and MLPA over full karyotype include substantially reduced turnaround time and automation and batching of samples resulting in reduced cost per sample. FISH is not amenable to automation and remains more costly than PCR-based techniques.
3. The main disadvantage of current RAD methods is the failure to detect chromosome aberrations that are not aneuploid for the targeted chromosomes. Some of these aberrations are predicted to have clinical morbidity.
4. In the future, RAD alone may be a suitable tool for prenatal diagnosis for a subset of women undergoing invasive testing solely for an increased risk of fetal aneuploidy. It would not be suitable for those women with other risk factors such as structural fetal abnormalities on ultrasound or a personal or family history of a chromosomal rearrangement (such as a balanced translocation). These women should always have a full cytogenetic karyotype analysis.
5. The introduction of new techniques for prenatal diagnosis should include analysis of benefits versus risks and cost effectiveness. RAD may be a suitable alternative to full karyotype when amniocentesis is undertaken primarily to exclude fetal aneuploidy. However, a counterargument is that an invasive procedure puts a pregnancy at risk, thereby justifying the most "complete" test available. It is likely that further development of prenatal molecular techniques will allow for improved detection of more chromosomal disorders at lower cost.

## Abstract

**Objective:** To review the molecular genetic techniques currently available for rapid prenatal diagnosis of fetal aneuploidy, as well as those still under investigation.

**Options:** Limited to introductory discussion of rapid aneuploidy detection methods.

**Evidence:** Medline was searched for articles related to the topic that were published after 1992. This document represents an abstraction of the information.

**Values:** This update was prepared by the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada approved by the Executive and Council of the Society of Obstetricians and Gynaecologists of Canada.

**Benefits, Harms, and Costs:** This update provides information about methods of rapid aneuploidy detection using molecular

**Key Words:** Prenatal diagnosis, genetic screening, chromosome aberrations, aneuploidy, fluorescence in situ hybridization, polymerase chain reaction

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## INTRODUCTION

The Canadian Task Force on Preventive Health Care recommends offering prenatal screening for chromosome abnormalities, in particular Down syndrome, to allow women to make reproductive choices.<sup>1</sup> Invasive diagnostic testing by amniocentesis or chorionic villus sampling is associated with a procedure-related pregnancy loss rate of up to 0.5%, and public health resources are limited. Therefore, new guidelines suggest that eligibility for invasive testing should be determined by an assessment of an individual woman's chance of having a child with Down syndrome, on the basis of non-invasive screening tests.<sup>2</sup> In some situations, invasive testing is warranted for reasons other than detection of Down syndrome and trisomies 13 and 18. For example, the risk of other types of chromosome abnormalities, (deletions or duplications) is increased when structural fetal abnormalities are seen on ultrasound or when a parent has a structural chromosome rearrangement predisposing to chromosomally abnormal offspring.

A full G-banded karyotype is the current standard of care for women undergoing invasive testing because of an increased risk for a fetal chromosome abnormality, often defined as greater than 1/300, which is close to that of procedure-related pregnancy loss. Karyotype is virtually 100% sensitive and specific for the detection of the autosomal trisomies 13, 18, and 21 and the sex chromosome aneuploidies, which together constitute the majority of abnormal fetal chromosome constitutions and which are correlated with advanced maternal age.<sup>3</sup> In addition, full karyotype has the advantage of detecting other chromosome aberrations, both numerical (triploidy and extra structurally abnormal chromosomes) and structural (deletions, translocations, inversions, and insertions), at a resolution of approximately 10 million DNA base-pairs. Such chromosome aberrations are unrelated to maternal age or abnormal results of biochemical screening tests and are often picked up incidentally or following detection of structural fetal abnormalities on ultrasound.<sup>4,5</sup>

Karyotype does have a number of limitations.<sup>6</sup> Because of the time required for cell culture and harvesting, as well as the labour-intensive process of analysis, full karyotype takes approximately 7 to 14 days. Although the laboratory cost varies by centre, a full karyotype a prenatal sample is

approximately Can\$500, including supplies and technician time (J. Chernos, personal communication, June 2006). Considerable effort has therefore gone into developing robust molecular genetic techniques that do not require cell culture prior to analysis and that are amenable to automation, therefore providing more rapid results at less expense to the health care system. The main disadvantage of such techniques is that they fail to detect chromosome aberrations other than aneuploidy for the targeted chromosomes, and some of these are clinically significant.

## METHODS OF RAPID ANEUPLOIDY DETECTION

Currently, two methods of RAD have been validated for routine use in prenatal diagnosis, generally in addition to, rather than replacing, karyotype: FISH and QF-PCR. MLPA is a newer technology established for other indications; it is still under investigation for use in prenatal diagnosis.

### Fluorescence in Situ Hybridization

**Technique:** FISH uses a fluorescently labelled probe targeted to a unique sequence of DNA where it selectively binds.<sup>7</sup> For prenatal samples, FISH is done on uncultured, interphase cells. For purposes of RAD, the probes used are specific for chromosomes 13, 18, 21, X, and Y. Samples are visualized using a microscope; the number of fluorescent signals per cell indicates the number of copies of the targeted chromosome. Standard practice is to score 100 cells to exclude mosaicism at a level of greater than 10% to 15%, a level similar to that of full karyotype.

**Clinical uses:** Interphase FISH has been used extensively in the UK and Europe for RAD in conjunction with full karyotype.<sup>8,9</sup> In most Canadian centres, FISH is undertaken as an add-on test for selected pregnancies deemed to be at very high risk for chromosome abnormalities, or in cases of advanced gestational age, in order to provide more rapid results to couples faced with time-sensitive decisions about pregnancy termination. FISH is also/can also be used for targeted diagnosis of several common chromosomal microdeletions associated with structural fetal abnormalities (such as 22q11 deletion syndrome in the presence of certain cardiac defects) or when parents have already had a child affected with a specific microdeletion syndrome.

**Advantages and limitations:** In a UK Health Technology Assessment, FISH was found to be virtually 100% sensitive and 100% specific for the detection of the targeted aneuploidies.<sup>10</sup> Another advantage is the capacity to detect triploidy. The main limitation of FISH, and the factor that prevents cost from being minimized, is its unsuitability for automation.<sup>10,11</sup> Analysis involves considerable time with a skilled technician. Maternal cell contamination can (rarely)

## ABBREVIATIONS

FISH	fluorescence in situ hybridization
MLPA	multiplex ligation-dependent probe amplification
QF-PCR	quantitative fluorescence polymerase chain reaction
RAD	rapid aneuploidy detection

interfere with interpretation. The addition of FISH to full karyotype approximately doubles the cost to \$1000 for both tests (J. Chernos, personal communication, June 2006).

### Quantitative Fluorescence Polymerase Chain Reaction

**Technique:** PCR is a well-established molecular genetic technique that selectively amplifies regions of genomic DNA on the basis of the binding of primers which are unique to that region. QF-PCR is a newer method that can be used to determine the copy number of a DNA sequence.<sup>11–13</sup> On DNA extracted from uncultured amniocytes or chorionic villi, QF-PCR amplifies polymorphic DNA markers specific for the chromosomes of interest (13, 18, 21, and sometimes X and Y). Fluorescently labelled primers bind to each target sequence and allow DNA polymerase to replicate the strand, synthesizing double-stranded DNA. Following amplification, the products are separated by size, using a capillary electrophoresis system. Computer-assisted measurement of the intensity of the fluorescent signal allows copy number to be determined for each target sequence and therefore each chromosome.

**Clinical uses:** QF-PCR is routinely used in the UK and several European countries for detection of the common autosomal aneuploidies and sex chromosome aneuploidies.<sup>14–17</sup> At present, it is not widely used in prenatal diagnosis in Canada.

**Advantages and limitations:** According to the UK Health Technology Assessment, QF-PCR is as reliable and precise as FISH and karyotype for targeted aneuploidies, with sensitivity and specificity of 95.65% and 99.97%, respectively.<sup>10</sup> In addition, it reliably detects triploidy. QF-PCR detects mosaicism at a level similar to that of full karyotype.<sup>18</sup> The problem of maternal cell contamination masking abnormal fetal karyotypes is also minimized, and in the rare event that results are equivocal, comparison with a maternal blood specimen can be used to make a determination.<sup>11</sup> A major advantage over FISH is amenability to automation and batching of samples, which lowers the cost per sample to around \$20.<sup>10</sup> However, most diagnostic laboratories use commercial kits to perform QF-PCR, which may increase the cost above this amount.

### Multiplex Ligation-Dependent Probe Amplification

**Technique:** MLPA is a new PCR-based technology that discriminates between copy numbers of specific sequences of DNA.<sup>19</sup> MLPA uses two-part probes of unique length that, when hybridized to adjacent target sequences on genomic DNA, can be joined together by the enzyme DNA ligase. This then allows all target sites to be amplified using a single primer pair that is complementary to the two free ends common to all probes. The products are run on a

capillary electrophoresis system and separated by size, so that each peak is the amplification product of a specific probe. Using a series of normalization calculations, copy number can be determined for each target sequence and therefore each chromosome.

**Clinical uses:** MLPA is an emerging technique that has been employed successfully by several independent groups for aneuploidy detection in prenatal diagnosis.<sup>20–23</sup> However, it is a new method that is not yet being used routinely by centres offering FISH or QF-PCR. MLPA has other clinical uses, including, as with FISH, the prenatal diagnosis of microdeletion syndromes. MLPA panels are also being developed to screen for dozens of such conditions (such as Prader-Willi and Angelman) that would otherwise not be detected prenatally.

**Advantages and limitations:** MLPA is proving to be a rapid, simple, and reliable method with cost comparable to QF-PCR.<sup>21</sup> Sensitivity of 100% and specificity of 99.8% for non-mosaic targeted aneuploidies have been reported, but these were based on a sample size of only 527 amniocenteses, a relatively small number. MLPA offers several potential advantages, including low cost and the ability to amplify multiple markers (up to 40) in one tube. Disadvantages include the inability to detect all cases of triploidy, unknown sensitivity for mosaicism, and the fact that assays can be time-consuming and difficult to develop. MLPA is still under investigation and not yet ready for widespread introduction into prenatal diagnosis.

### DISCUSSION

The benefits and limitations of RAD methods have been reviewed. Clearly, molecular genetics has an evolving role in the prenatal diagnosis of chromosomal disorders, as an adjunct to rather than as a replacement for cytogenetics. FISH and QF-PCR are known to, and MLPA is proving to, have sensitivity and specificity similar to full karyotype for the detection of fetal aneuploidy (for chromosomes 13, 18, and 21 and the sex chromosomes). Advantages of QF-PCR and MLPA over full karyotype include substantially reduced turnaround time, and automation and batching of samples resulting in reduced cost per sample. FISH is not amenable to automation and remains more costly than PCR-based techniques.

In most Canadian centres, the use of RAD (primarily FISH) is restricted to select high-risk cases to provide more rapid results to women faced with time-sensitive decisions about pregnancy termination. Eligibility for RAD is usually based on locally determined criteria that take into consideration the additional cost incurred, the likelihood of a positive result, and the effect on clinical management. For example, in some centres, FISH is available to women with a risk of

aneuploidy of 1/20 or higher or late gestation at presentation. In these circumstances, RAD is considered an add-on and full cytogenetic karyotyping is still recommended.

The issue of whether FISH or QF-PCR can stand alone in select circumstances is controversial<sup>5,13,17,24–27</sup>. Proponents suggest that RAD by QF-PCR, in particular, is a cost-effective approach and detects the vast majority of clinically significant chromosome abnormalities in screen-positive women by providing a rapid, accurate chromosome count with nearly 100% sensitivity for trisomy 13, 18, and 21 and monosomy X, without the need for full karyotype. Opponents argue that missing any chromosome abnormality, is not acceptable; they contend that intangible costs to disabled children and their parents outweigh any monetary cost savings afforded by RAD. It should be emphasized that in all cases when an abnormality is detected on ultrasound (including nuchal translucency measurement greater than 3.5 mm and fetal anomalies) or there is a positive genetic history, back-up culture with full karyotype confirmation would always be indicated.

Another argument in support of RAD alone is that it would avoid the unexpected or incidental identification of rare chromosomal abnormalities following invasive testing primarily for increased risk of aneuploidy (trisomies 13, 18, and 21). Chromosome abnormalities predicted to be of no, or uncertain, clinical significance can be problematic for genetic counselling and have the potential to cause parental anxiety, possibly leading to termination of otherwise wanted pregnancies. Although it is true that other chromosome abnormalities may be predicted (or have the potential) to be clinically significant, Ogilvie et al. point out that the rate of detection of non-aneuploid chromosome abnormalities is similar prenatally and postnatally in the general population.<sup>24</sup> Women usually are undergoing invasive testing primarily for increased risk of aneuploidy and are at no greater risk for non-aneuploid chromosome abnormalities than the unselected pregnant population.<sup>4</sup>

In prenatal diagnosis, as in other areas of medicine, progress is measured by the ability to offer *more* to patients over time (e.g., more screening options and greater availability of diagnostic tests). Although replacement of karyotype by RAD alone in some circumstances would mean offering less, not more, to these women, it is likely that molecular techniques will soon be the key to offering more for less. The optimization of techniques such as MLPA and microarrays (reviewed by Rickman et al.<sup>3,28</sup>) is leading to an increase in the number of conditions that can be tested for simultaneously, while competition within the biotechnology industry has brought considerable decreases in cost. It is conceivable that in the future, women undergoing amniocentesis will have the option of a single test that detects full or

segmental chromosomal aneuploidy, at a cost lower than is currently associated with full karyotype.

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### GLOSSARY

**Aneuploidy:** A chromosome number that is not an exact multiple of 23, usually resulting from a meiotic non-disjunction error in the production of gametes.

**Autosome:** Any chromosome other than the sex chromosomes.

**Chromosome:** A linear structure containing a single strand of DNA. A human normally has 46 chromosomes in 23 pairs.

**DNA hybridization:** The bonding of a labelled DNA probe to a complementary target sequence.

**Karyotype:** The chromosome constitution of an individual, or the photomicrograph of an individual's chromosomes, systematically arranged in 23 pairs.

**Monosomy:** The absence of a single chromosome.

**Mosaicism:** The presence of two or more genetically different cell lines in an individual or tissue.

**Numerical chromosome aberration:** A chromosome number that is not 46.

**Structural chromosome aberration:** A chromosome number of 46 in which segment(s) of chromosome(s) are missing (deleted), extra (inserted), or rearranged (translocated or inverted).

**Triploidy:** A chromosome number of 69 (three copies of each chromosome).

**Trisomy:** The presence of an extra chromosome.

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