Use of a DNA Method, QF-PCR, in the Prenatal Diagnosis of Fetal Aneuploidies

This Clinical Practice Guideline has been prepared by the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada (SOGC) and the Prenatal Diagnosis Committee of the Canadian College of Medical Geneticists (CCMG) and approved by the Executive and Council of the SOGC and the Board of Directors of the CCMG.

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Disclosure statements have been received from all members of the committees.

Abstract

Objective: To provide Canadian health care providers with current information on the use of quantitative fluorescent polymerase chain reaction (QF-PCR) or equivalent technology in the prenatal diagnosis of fetal chromosomal abnormalities.

Options: Over the last few decades, prenatal diagnosis of fetal chromosomal abnormalities has relied on conventional cytogenetic analysis of cultured amniocytes, chorionic villi, or fetal blood. In the last few years, the clinical validity of a newer technique, QF-PCR, to detect the common aneuploidies has been reported by a number of investigators. This technique has the advantage of providing rapid results for the diagnosis or exclusion of aneuploidy in chromosomes 13, 18, 21, X or Y. It is now possible to choose standard chromosome analysis or QF-PCR for the prenatal diagnosis of chromosomal abnormalities, or to perform both tests, depending on the clinical indication for testing. This document reviews the clinical utility of QF-PCR and makes recommendations for its use in the care of Canadian patients.

Evidence: Medline and PubMed were searched for articles published in English between January 2000 and December 2010 that presented data on the use of QF-PCR versus standard cytogenetic analysis of prenatal samples. A second search was done to identify publications in English that provided results of cytogenetic analysis performed on prenatal samples for women at an increased risk of fetal aneuploidy because of maternal age, abnormal prenatal screening results, or fetal soft ultrasound markers suggestive of an increased risk of aneuploidy.

Key Words: Quantitative fluorescent polymerase chain reaction (QF-PCR), fetal chromosomal abnormalities, prenatal diagnosis

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Publications were included if they provided detailed information on the abnormalities detected, regardless of whether or not rapid aneuploidy screening was undertaken.

Results were restricted to systematic reviews, randomized controlled trials, and relevant observational studies. Grey (unpublished) literature was identified through searching the websites of health technology assessment and health technology assessment-related agencies, clinical practice guideline collections, clinical trial registries, and national and international medical specialty societies.

Values: The quality of evidence was rated using the criteria described in the Report of the Canadian Task Force on Preventive Health Care (Table 1).

Benefits, harms, and costs: This guideline promotes the use of a rapid aneuploidy DNA test for women at increased risk of having a pregnancy affected by a common aneuploidy. This will have the benefit of providing rapid and accurate results to women at increased risk of fetal Down syndrome, trisomy 13, trisomy 18, sex chromosome aneuploidy or triploidy. It will also promote better use of laboratory resources and reduce the cost of prenatal diagnosis. However, a small percentage of pregnancies with a potentially clinically significant chromosomal abnormality will remain undetected by QF-PCR but detectable by conventional cytogenetics.

Recommendations

1. QF-PCR is a reliable method to detect trisomies and should replace conventional cytogenetic analysis whenever prenatal testing is performed solely because of an increased risk of aneuploidy in chromosomes 13, 18, 21, X or Y. As with all tests, pretest counselling should include a discussion of the benefits and limitations of the test. In the initial period of use, education for health care providers will be required. (II-2A)

2. Both conventional cytogenetics and QF-PCR should be performed in all cases of prenatal diagnosis referred for a fetal ultrasound abnormality (including an increased nuchal translucency measurement > 3.5 mm) or a familial chromosomal rearrangement. (II-2A)

3. Cytogenetic follow-up of QF-PCR findings of trisomy 13 and 21 is recommended to rule out inherited Robertsonian translocations. However, the decision to set up a back-up culture for all cases that would allow for traditional cytogenetic testing if indicated by additional clinical or laboratory information should be made by each centre offering the testing according to the local clinical and laboratory experience and resources. (III-A)

4. Other technologies for the rapid detection of aneuploidy may replace QF-PCR if they offer a similar or improved performance for the detection of trisomy 13, 18, 21, and sex chromosome aneuploidy. (III-A)


ABBREVIATIONS

FISH fluorescence in situ hybridization

QF-PCR quantitative fluorescent polymerase chain reaction

INTRODUCTION

Over the last few decades, prenatal diagnosis of fetal chromosomal abnormalities has relied on conventional cytogenetic analysis of cultured amniocytes, chorionic villi, or fetal blood. FISH on uncultured amniocytes or chorionic villi has been added in some cases for the rapid detection of aneuploidy in chromosomes 13, 18, 21, X and Y. In the last few years, the clinical utility of using QF-PCR to detect common aneuploidies has been reported by a number of investigators. These new molecular techniques for the prenatal detection of chromosomal aneuploidy are reviewed in more detail in a Society of Obstetricians and Gynaecologists of Canada technical update.1 QF-PCR is a PCR-based technique that consists of amplifying polymorphic markers located on the chromosomes of interest to determine the number of copies of those chromosomes present per cell. The advantages of QF-PCR are that it requires a small sample and allows automation of the procedure, providing a rapid turnaround time at a lower cost than conventional cytogenetics. Moreover, diagnostic testing with QF-PCR eliminates the unexpected or incidental identification of rare chromosomal abnormalities of uncertain significance, whereas G-banding karyotyping can yield results with a low predictive value for abnormal phenotype because of the detection of mosaicism and de novo balanced rearrangements.2 Some authors have suggested that because of its high sensitivity and specificity for the detection of the common aneuploidies, QF-PCR should replace conventional chromosome analysis as the method of prenatal diagnostic testing in pregnancies at an increased risk for fetal trisomy 18 or trisomy 21 because of maternal age or an abnormal prenatal screening test result; conventional chromosome analysis would continue to be used for cases determined to be at increased risk for fetal chromosomal abnormality because of a fetal structural anomaly detected by ultrasound.3-5

PERFORMANCE OF QF-PCR IN DETECTING NON-MOSAIC TRISOMY 13, 18, 21, TRIPLOIDY, AND SEX CHROMOSOMAL ANEUPLOIDY

To assess the performance of QF-PCR in detecting non-mosaic trisomy 13, 18, 21, triploidy, and sex chromosomal aneuploidies, data were collected from the relevant publications to answer three main questions:

1. What percentage of cases cannot be reported because either the PCR failed or the results are inconclusive because of maternal cell contamination?
2. Of the cases reported, are there any false-positive diagnoses defined as a pregnancy with a normal karyotype but in which the QF-PCR result was reported to be abnormal?

3. Of the cases reported, are there any false-negative diagnoses by QF-PCR of non-mosaic trisomy 13, 18, 21, triploidy, and sex chromosomal aneuploidies?

The data extracted from relevant publications are presented in Table 2.

In total, 79 556 prenatal samples were analyzed. A small proportion (1.3%) of samples could not be reported, predominantly because of maternal cell contamination that caused an inconclusive result. In contrast, cytogenetic analysis was unsuccessful because of culture failure of amniocytes in 0.12% to 0.3% of cases. The performance of the QF-PCR test is dependent on the number of markers analyzed per chromosome. In the original studies, a number of cases remained uninformative for one or more chromosomes. This problem seems to have been resolved by the typing of additional markers, as shown in a large 2009 study. Enough polymorphic markers must be typed for each chromosome of interest to ensure accurate determination of copy number.

Among all studies reviewed, no false-positive diagnoses were made. All cases found to be abnormal by QF-PCR were abnormal on conventional cytogenetic analysis, although in 5 cases of sex chromosomal aneuploidy, an incorrect diagnosis was made. Four of these cases were interpreted as 47,XXX by QF-PCR but were diagnosed by cytogenetic analysis as mosaic 45,X/46,XX, and one case was interpreted as 47,XXX by QF-PCR but was mosaic 47,XXX/46,XX by cytogenetic analysis. Among the 79 556 prenatal samples compiled in this review, there were 2674 cases of non-mosaic trisomy 13, 18, or 21 or triploidy. Two false-negative cases of trisomy were reported in one study that analyzed only 2 markers per chromosome. There were actually no other false-negatives, because the 7 cases not diagnosed by QF-PCR were not reported as normal but were uninformative because of maternal cell contamination.

There were 328 cases of non-mosaic sex chromosomal aneuploidy. Fifteen cases were missed. The accuracy of the diagnosis of sex chromosomal aneuploidy appears to depend on the panel of X chromosome markers used in the analysis by each investigator. Cirigliano et al. missed one case early in their series and none after additional markers were added to the panel. This suggests that with the currently available panels non-mosaic sex chromosomal aneuploidy cases can be reliably detected.

In summary, 98.7% of samples analyzed by QF-PCR have conclusive results, with no false-positive or false-negative diagnosis of non-mosaic trisomy for chromosomes 13, 18, or 21 or triploidy. Although no false-positive diagnosis was made for sex chromosomal aneuploidy, some earlier studies reported false-negative results. Currently, these would seem less likely to occur because of the implementation of additional polymorphic markers.

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**Table 1. Key to evidence statements and grading of recommendations, using the ranking of the Canadian Task Force on Preventive Health Care**

<table>
<thead>
<tr>
<th>Quality of evidence assessment*</th>
<th>Classification of recommendations†</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: Evidence obtained from at least one properly randomized controlled trial</td>
<td>A. There is good evidence to recommend the clinical preventive action</td>
</tr>
<tr>
<td>II-1: Evidence from well-designed controlled trials without randomization</td>
<td>B. There is fair evidence to recommend the clinical preventive action</td>
</tr>
<tr>
<td>II-2: Evidence from well-designed cohort (prospective or retrospective) or case-control studies, preferably from more than one center or research group</td>
<td>C. The existing evidence is conflicting and does not allow to make a recommendation for or against use of the clinical preventive action; however, other factors may influence decision-making</td>
</tr>
<tr>
<td>II-3: Evidence obtained from comparisons between times or places with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of treatment with penicillin in the 1940s) could also be included in this category</td>
<td>D. There is fair evidence to recommend against the clinical preventive action</td>
</tr>
<tr>
<td>III: Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees</td>
<td>E. There is good evidence to recommend against the clinical preventive action</td>
</tr>
<tr>
<td></td>
<td>F. There is insufficient evidence (in quantity or quality) to make a recommendation; however, other factors may influence decision-making</td>
</tr>
</tbody>
</table>

*The quality of evidence reported in these guidelines has been adapted from The Evaluation of Evidence criteria described in the Canadian Task Force on Preventive Health Care.†Recommendations included in these guidelines have been adapted from the Classification of Recommendations criteria described in the Canadian Task Force on Preventive Health Care.
Table 2. Performance of QF-PCR

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number and type of specimens</th>
<th>Sample failure/not tested or UI, %</th>
<th>Number of cases detected / number of actual non-mosaic autosomal trisomies and triploidies</th>
<th>False positives for trisomy or triploidy</th>
<th>Number of cases detected / number of actual non-mosaic sex chromosomal aneuploidies</th>
<th>False positives for sex chromosomal aneuploidy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levet et al. 2001&lt;sup&gt;6&lt;/sup&gt;</td>
<td>5097 AF</td>
<td>0.1 / 2</td>
<td>89/89</td>
<td>0</td>
<td>16/20</td>
<td>0</td>
</tr>
<tr>
<td>Schmidt et al. 2001&lt;sup&gt;7&lt;/sup&gt;</td>
<td>662 AF</td>
<td>0 / 0</td>
<td>14/15</td>
<td>0</td>
<td>5/5*</td>
<td>0</td>
</tr>
<tr>
<td>Bili et al. 2002&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1020 AF</td>
<td>0 / 1.2</td>
<td>16/19</td>
<td>0</td>
<td>0/0</td>
<td>0</td>
</tr>
<tr>
<td>Mann et al. 2004&lt;sup&gt;8&lt;/sup&gt;</td>
<td>7720 (6147AF, 1552 CVS, 21 FBS)</td>
<td>0.09 / 2.1</td>
<td>437/437</td>
<td>0</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Caine et al. 2005&lt;sup&gt;10&lt;/sup&gt;</td>
<td>10253 AF</td>
<td>2.9</td>
<td>429/429</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Brown et al. 2006&lt;sup&gt;11&lt;/sup&gt;</td>
<td>687 AF</td>
<td>0 / 2.5</td>
<td>14/14</td>
<td>0</td>
<td>1/3</td>
<td>0</td>
</tr>
<tr>
<td>Kozlowski et al. 2006&lt;sup&gt;12&lt;/sup&gt;</td>
<td>4692 AF</td>
<td>0.06 / 0.15</td>
<td>71/73</td>
<td>2 cases missed at the beginning when only two markers per chr used.</td>
<td>0</td>
<td>2/8</td>
</tr>
<tr>
<td>Kagan et al. 2007&lt;sup&gt;4&lt;/sup&gt;</td>
<td>3854 AF</td>
<td>None reported</td>
<td>202/202</td>
<td>0</td>
<td>14/14</td>
<td>0</td>
</tr>
<tr>
<td>Onay et al. 2008&lt;sup&gt;13&lt;/sup&gt;</td>
<td>576 AF</td>
<td>0 / 2.9</td>
<td>15/15</td>
<td>0</td>
<td>4/4</td>
<td>0</td>
</tr>
<tr>
<td>Putzova et al. 2008&lt;sup&gt;14&lt;/sup&gt;</td>
<td>2906 (142 CVS, 2764 AF)</td>
<td>0 / 0.26 for AF</td>
<td>110/110</td>
<td>0</td>
<td>20 / 20</td>
<td>0</td>
</tr>
<tr>
<td>Cirigliano et al. 2009&lt;sup&gt;15&lt;/sup&gt;</td>
<td>37544 AF, 4687 CVS</td>
<td>0.05 / 0.82</td>
<td>1287/1290†</td>
<td>3 cases were UI</td>
<td>265/267†‡</td>
<td>1 abnormality missed; 1 UI MCC</td>
</tr>
</tbody>
</table>

AF: amniotic fluid; chr: chromosome; CVS: chorionic villus sampling; FBS: fetal blood sample; MCC: maternal cell contamination; UI: reported as uninformative because of MCC or uninformative markers.

*1 case with a cytogenetic diagnosis of 47,XXY/46,XX was diagnosed by QF-PCR as 47,XXY.

†4 cases of 48,XXY+21, 3 cases of 48,XXY+18 and 1 case of 68,XX are included in the total numbers of both autosomal aneuploidies/triploidies and sex chromosomal aneuploidies.

‡4 cases with a cytogenetic diagnosis of 45,X/46,XX were diagnosed by QF-PCR as 47,XXY.

Residual Risk of a Chromosomal Abnormality Given a Negative QF-PCR Result

Although QF-PCR has been shown to be a reliable method to detect non-mosaic cases of trisomy 13, 18, and 21 and sex chromosomal aneuploidy, it is less reliable in the detection of mosaic cases. Cirigliano et al. reported diagnosis of 45 out of the 72 mosaic cases in their series.<sup>15</sup> Furthermore, QF-PCR is not designed to detect trisomies other than 13, 18, and 21 and will not detect most unbalanced chromosomal abnormalities, nor will it detect balanced rearrangements. Therefore, a patient with a negative QF-PCR result has a residual risk of having a pregnancy with a chromosomal abnormality detectable on conventional chromosome analysis. To calculate this risk in pregnancies having fetal chromosome analysis for abnormal prenatal screening, maternal age or a soft marker of aneuploidy on ultrasound, prenatal series that reported all prenatal cytogenetic results were included in this analysis, regardless of whether QF-PCR was performed. When it was not performed, it was assumed that all cases of non-mosaic trisomy 13, 18, 21, triploidy, and sex chromosomal aneuploidy would be detected by QF-PCR. The residual risk was defined as the number of cases having a cytogenetic abnormality not detected by QF-PCR over the number of cases with normal results.
on QF-PCR.\textsuperscript{12} This residual risk was calculated for chromosomal abnormalities associated with a low, unknown, or high risk of abnormal outcome. Chromosomal abnormalities associated with a low risk included de novo apparently balanced rearrangements and cases in which parents were unavailable for testing. Chromosomal abnormalities of unknown risk included mosaic cases and de novo marker chromosomes. Chromosomal abnormalities associated with a high risk included other trisomies and unbalanced structural rearrangements. Inherited balanced chromosomal rearrangements or markers were considered to confer no increased risk and were included in the total residual risk. Table 3 provides the compiled data. Given a negative QF-PCR result, the residual risk for a chromosomal abnormality of low, high, or unknown risk is 0.44% or 1 in 227. If inherited balanced chromosomal rearrangements or markers are included, the risk is 0.73% (1 in 137).

### OTHER TECHNOLOGIES

FISH has been shown to be an accurate method to detect fetal aneuploidy, and commercial kits are available to test for trisomy 13, 18, 21, and sex chromosome aneuploidies. However, QF-PCR has the advantage over FISH of being much less expensive and of allowing the simultaneous processing of much larger numbers of samples.\textsuperscript{20} Although not as extensively studied as QF-PCR, multiplex ligation-dependent probe amplification has been shown to be a reliable rapid aneuploidy detection method, with 100% sensitivity and 100% specificity for the diagnosis of aneuploidies of chromosomes X, Y, 13, 18, and 21 in a prospective cohort study of 4585 women.\textsuperscript{21} This technology does not detect cases of triploidy. However, such cases would be expected to have associated ultrasound findings, and conventional cytogenetic analysis would be indicated.

The field of molecular genetics is rapidly evolving. Although currently QF-PCR would be considered the technology of choice for rapid aneuploidy detection, it is likely that other platforms will be developed that may have the capacity to detect additional abnormalities such as cases of segmental aneuploidies. For example, a bead array approach with bacterial artificial chromosomes—containing microbeads with probes for the aneuploidies and microdeletion syndromes has been developed and validated on a small number of samples. Results indicate that this approach is a rapid and reliable test for common aneuploidies and microdeletions.\textsuperscript{22}

### Recommendations

1. QF-PCR is a reliable method to detect trisomies and should replace conventional cytogenetic analysis whenever prenatal testing is performed solely because of an increased risk of aneuploidy in chromosomes 13, 18, 21, X or Y. As with all tests, pretest counselling should include a discussion of the benefits and limitations of the test. In the initial period of use, education for health care providers will be required. (II-2A)

2. Both conventional cytogenetics and QF-PCR should be performed in all cases of prenatal diagnosis referred for a fetal ultrasound abnormality (including an increased nuchal translucency measurement > 3.5 mm) or a familial chromosomal rearrangement. (II-2A)
3. Cyto genetic follow-up of QF-PCR findings of trisomy 13 and 21 is recommended to rule out inherited Robertsonian translocations. However, the decision to set up a back-up culture for all cases that would allow for traditional cytogenetic testing if indicated by additional clinical or laboratory information should be made by each centre offering the testing according to the local clinical and laboratory experience and resources. (III-A)

4. Other technologies for the rapid detection of aneuploidy may replace QF-PCR if they offer a similar or improved performance for the detection of trisomy 13, 18, 21, and sex chromosome aneuploidy. (III-A)

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.

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

DNA: The molecule that encodes genes.

FISH: Fluorescence in situ hybridization.

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 2 or more genetically different cell lines in an individual or tissue.

Numerical chromosome aberration: A chromosome number which 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 (3 copies of each chromosome).

Trisomy: The presence of an extra chromosome.

REFERENCES


3. Nicolini U, Lalatta F, Natacci F, Curcio C, Bui T-H. The introduction of Robertsonian translocations. However, the decision to set up a back-up culture for all cases that would allow for traditional cytogenetic testing if indicated by additional clinical or laboratory information should be made by each centre offering the testing according to the local clinical and laboratory experience and resources. (III-A)

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