Detecting Aneuploidy of Chromosomes 13, 18, 21, X, And Y With QF-PCR (Reference – 2013.03.004)

Notice Of Assessment

April 2014
1 GENERAL INFORMATION

1.1 Requestor: Montreal Children’s Hospital (MCH-MUHC)

1.2 Application for Review Submitted to MSSS: September 24, 2013

1.3 Application Received by INESSS: November 1, 2013

1.4 Notice Issued: February 28, 2014

Note
This notice is based on the scientific and commercial information submitted by the requestor and on a complementary review of the literature according to the data available at the time that this test was assessed by INESSS.

2 TECHNOLOGY, COMPANY, AND LICENCE(S)

2.1 Name of the Technology
Quantitative fluorescence polymerase chain reaction (QF-PCR).

2.2 Brief Description of the Technology, and Clinical and Technical Specifications
PCR is a well-established molecular genetic technique that selectively amplifies certain regions of genomic DNA on the basis of the binding of primers specific to that region. QF-PCR amplifies a specific segment of the chromosome of interest (13, 18, 21, X, and Y) in a DNA sample from amniotic fluid obtained by amniocentesis or chorionic villi samples, or from other tissue or blood samples from newborns or adults. The primers labelled by fluorescence bind to each target sequence, thus allowing DNA polymerase to replicate the fragment (strand) and synthesize double-stranded DNA. The products are run on a capillary electrophoresis system and separated by size, so that each peak is the product of the amplification of a specific probe. The fluorescent signal intensity is measured with a computer system to determine the copy number for each target sequence, and, therefore, each chromosome [Sparkes et al., 2008]. The results of this technique are qualitative (change in chromosome structure) and quantitative (number of chromosomes).

2.3 Company or Developer
The requestor uses the Aneufast™ kit developed by Molgentix SL (Spain), a parent company of Genomed, to detect aneuploidy of chromosomes 13, 18, 21, X, and Y.

2.4 Licence(s): Not applicable.

2.5 Patent, If Any
The Aneufast™ kit was patented in the United States (patent numbers 6,008,379; 6,020,481; 6,221,604; and 6,303,775 according to the user’s manual revised in August 2011).

2.6 Approval Status (Health Canada, FDA)
The Aneufast™ kit has not been approved by Health Canada. However, according to the requestor, it is used in various Canadian laboratories (Children’s Hospital of Eastern Ontario [CHEO] in Ottawa, North York General Hospital and Mount Sinai Hospital in Toronto, as well as other laboratories in London, Halifax, and St. John’s).
2.7 Weighted Value: 178

3 CLINICAL INDICATIONS, PRACTICE SETTINGS, AND TESTING PROCEDURES

3.1 Targeted Patient Group

The test targets the fetuses of pregnant women who are at a high risk of chromosomal abnormalities, as indicated by their prenatal screening test results or as a result of maternal age or family history [Langlois et al., 2011; Dickinson et al., 2009; Conrad et al., 2005]. The test is also used for postnatal diagnosis of chromosomal aneuploidies [Saadi et al., 2010].

3.2 Targeted Disease(s)

Based on the information available on Orphanet, trisomy 13 or Patau syndrome (affecting 1/10,000 pregnancies in Quebec), is generally suspected during pregnancy, when ultrasound reveals abnormalities, and an estimated 95% of affected fetuses die before birth. Half of live-born children die during the first month, and 90% during the first year as a result of cardiac, renal, or neurological complications. Those who survive to adulthood are generally affected by partial trisomy or by mosaicism, but may not have any major brain malformations.

Trisomy 18 (affecting 1/4,000 pregnancies in Quebec) may also be detected during pregnancy through ultrasound findings or abnormal results of serum markers for trisomy 21 screening. It is also a serious disease that causes in utero death in 95% of affected fetuses. As is the case with trisomy 13, the majority of children with the disease die before the age of 1 year, and the rare cases who live longer have partial trisomy or a mosaicism.

The Trisomy 21 Prenatal Screening Program of Québec (PODPT21) allows high-risk pregnancies to be identified using serum and sonographic markers. When a high risk case is identified, an amniocentesis is performed to confirm the diagnosis with fetal karyotyping. Trisomy 21 is a common aneuploidy (affecting 1/700 births in Quebec) with an increased risk with increasing maternal age as well as with family history. Children with the condition have distinct physical features, developmental delays, varying degrees of intellectual impairment, and various malformations.

Klinefelter syndrome in men comprises a group of chromosomal abnormalities characterized by the presence of at least one extra X chromosome in the male karyotype (46,XY) (e.g., 47,XXY; 48,XXXY, etc.). If the diagnosis has not been confirmed during the prenatal period by a cytogenetic test, the children undergo postnatal cytogenetic testing when clinical signs such as developmental delay, hypospadias, micropenis, or cryptorchidia are present. In children, clinical signs vary with age, and, in adults, the syndrome is generally diagnosed as a result of a sterility problem or breast cancer. The extent of the effect on the physical and cognitive phenotype is directly proportional to the number of extra X chromosomes.

Trisomy X (XXX) is an abnormality characterized by the presence of an extra X chromosome in the female karyotype (46,XX). It is the most common female chromosomal abnormality. Thirty percent of cases are associated with advanced maternal age. If trisomy X is not diagnosed during the prenatal period, the postnatal diagnosis is guided by clinical signs such as developmental delay, hypotonia.

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31 Verloes A. Trisomy 13 [website]. Available at: http://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=EN&Expert=3378.0 (viewed December 20, 2013).
32 Verloes A. Trisomy 18 [website]. Available at: http://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=EN&Expert=3380.0 (viewed December 20, 2013).
learning disabilities, behavioural problems, or premature ovarian failure.\textsuperscript{35}

Turner syndrome is often detected by ultrasound during pregnancy, but forms lacking the associated malformations are discovered through karyotyping, which is often performed in cases of advanced maternal age. The syndrome is characterized by the absence of an X chromosome (45,X). However, the majority of cases are caused by the presence of a mosaicism (45,X/46,XX) or an abnormal X chromosome. Manifestations of the disease may include ovarian insufficiency, bone abnormalities, lymphedema, and deafness, as well as cardiovascular, thyroid, and gastrointestinal abnormalities.\textsuperscript{36}

3.3 Number of Patients Targeted

According to the requestor’s estimates, the number of tests may vary depending on the chosen procedures:

- approximately 300 samples per year from RUIS McGill alone if the test is performed in cases of pregnancies presenting a very high risk of aneuploidy;
- between 800 and 900 cases per year for RUIS McGill alone (3,000 and 3,500 per year for the entire province) if it is used as a diagnostic test for pregnant women undergoing a amniocentesis to detect aneuploidy prior to karyotyping.

3.4 Medical Specialties and Other Professions Involved

All health care professionals working in obstetrics (general practitioners in perinatology, obstetrician-gynecologists, midwives, nurses in perinatology), medical genetics, molecular genetics, and genetic counselling.

3.5 Testing Procedure

The test is performed using fetal DNA from fetal cells in amniotic fluid specimens obtained through amniocentesis or from chorionic villus sampling. These two techniques are performed under sterile conditions and with ultrasound guidance.\textsuperscript{37} In some cases, a sample of the mother’s buccal cells must also be collected and tested.

In postnatal testing, samples are taken from the blood or tissue of newborns or adults.

4 TECHNOLOGY BACKGROUND

4.1 Nature of the Diagnostic Technology: Substitutive or complementary.

4.2 Brief Description of the Current Technological Context

In Quebec, the PQDPT21 gives all pregnant women the opportunity to undergo prenatal screening with integrated biochemical testing, with or without a nuchal translucency ultrasound (depending on availability). When the risk of aneuploidy is high, as indicated by the screening results or as a result of the pregnant woman’s age or family history, an amniocentesis for fetal karyotype is offered to confirm the diagnosis. The sensitivity and specificity approach 100% for this test; the rare errors that may occur are attributable to the quality of the sample.

Karyotyping is also indicated for postnatal diagnosis of these abnormalities or for prenatal diagnosis in cases of a potential risk of other chromosomal abnormalities that cannot be detected by the PQDPT21.


Rapid tests can provide a diagnosis within a shorter time frame than karyotyping (2 to 3 weeks). These tests are: QF-PCR, fluorescence in situ hybridization (FISH), and multiplex ligation-dependent probe amplification (MLPA). The diagnostic performance of FISH and QF-PCR is equivalent to that of karyotyping (gold standard). However, unlike karyotyping, they can detect only a few aneuploidies and are not recommended for women in whom ultrasound examinations reveal risk factors such as structural abnormalities or for women with a family history of chromosomal abnormalities. QF-PCR is more cost-effective than FISH, and, unlike the latter, it can be automated [Sparkes et al., 2008].

4.3 Brief Description of the Advantages Cited for the New Technology

The QF-PCR method can provide results within 48 to 72 hours, unlike karyotyping, which requires 2 to 3 weeks [Langlois et al., 2011]. This shorter time frame can help reduce parents’ anxiety [Langlois et al., 2011; Badenas et al., 2010] and provide them with the genetic counselling they will need to make a decision if a chromosomal anomaly is identified [Dickinson et al., 2009]. QF-PCR is a simple, automatable technique that is more cost-effective than karyotyping or FISH and that allows the simultaneous processing of a larger number of samples than FISH [Langlois, et al., 2011; Baig et al., 2010; Conrad et al., 2005].

4.4 Cost of Technology and Options

While an economic analysis was not performed, a cost-effectiveness study was published by a Quebec team that compared the various screening strategies for trisomy 21. Of the screening strategies for trisomy 21, QF-PCR was more cost-effective ($24,084 for each case of trisomy 21 detected) than karyotyping ($27,898 for each case detected) [Gekas et al., 2011].

5 EVIDENCE

5.1 Clinical Relevance

5.1.1 Other Tests Replaced

QF-PCR could replace FISH for the detection and diagnosis of aneuploidies of chromosomes 13, 18, 21, X, and Y, and could therefore be used as a diagnostic test prior to karyotyping. In the case of negative, equivocal, or uncertain results, or of technical failure, karyotyping could confirm the diagnosis [Papoulidis et al., 2012; Badenas et al., 2010].

5.1.2 Diagnostic Value

This test has significant benefits, as it can rapidly produce results indicating chromosomal aneuploidies, in 48 to 72 hours rather than in the 2 to 3 weeks required by karyotyping. The rapidity of the test reduces the duration of the mother’s anxiety, as it allows a diagnosis to be made at a much earlier gestational age.

Treatment Modifications Based on Test Results

In Quebec, pregnant women or couples who receive a result confirming the diagnosis of a chromosomal abnormality may receive genetic counselling to help them prepare for the birth of the baby or consider terminating the pregnancy.38 In the postnatal period, the management process will require multidisciplinary consultations with the parents.

5.1.3 Adverse Effects

The technique used for amniotic fluid analysis (amniocentesis) or chorionic villus sampling (in certain situations) has some adverse effects. It is an invasive procedure that involves a risk of fetal loss. While

the risk varies according to the expertise of the professional or centre performing the test, it is nevertheless present, even in centres that have developed the greatest expertise (1/200 to 1/400 for amniocentesis in the second trimester, and up to 5% with chorionic villus sampling in the first trimester).

In the postnatal period or in adults, the inconveniences of blood testing or tissue sampling are minimal. However, given that the sampling method is the same for karyotyping and FISH, the risk involved is identical for the three tests.

A false-negative test may mistakenly reassure parents and leave them unprepared for the birth of a baby with a chromosomal abnormality. However, a false-positive result may lead to the termination of a pregnancy when the fetus is not affected [Baig et al., 2010; Nicolini et al., 2004].

### 5.2 Clinical Validity

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>PRESENCE</th>
<th>ABSENCE</th>
<th>NOT APPLICABLE</th>
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<tbody>
<tr>
<td>Sensitivity</td>
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<tr>
<td>Specificity</td>
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<td>Positive predictive value (PPV)</td>
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<td>Negative predictive value (NPV)</td>
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<td>Likelihood ratio (LR)</td>
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<td>ROC curve</td>
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<tr>
<td>Accuracy</td>
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Table 1 shows the clinical validity of QF-PCR. In general, the sensitivity is excellent (> 90%), except in one study that reports 75.3% [Papoulidis et al., 2012]. The specificity approaches 100% [Moftah et al., 2013; Papoulidis et al., 2012; Cirigliano et al., 2009; Kagan et al., 2007]. The accuracy also approaches 100% in the same studies. The positive predictive values (PPV) and negative predictive values (NPV) in these studies generally show excellent results (PPV of 91% to 100% and NPV of 86% to 99.7%), except in the study by Papoulidis et al. [2012], which reports a PPV of 75.3% and an NPV of 99% (Table 1).

The Ontario study by Speevak et al. [2011] presents the results of QF-PCR on 4,176 samples of amniotic fluid obtained over the period of one year. Karyotyping was the reference method. QF-PCR detected 98.5% of the abnormalities that have significant clinical consequences and 94.6% of the abnormalities detected by karyotyping. The detection rate was similar to that indicated in other studies [Cirigliano et al., 2009; Baig et al., 2010].

Birch et al. [2013] published a poster on the results of the clinical validation of QF-PCR at the McGill University Health Centre (MUHC). In total, 142 amniotic fluid samples were analyzed by QF-PCR and karyotyping. The sensitivity, specificity, and positive predictive value are 100%. The negative predictive value is 98.5%.

The clinical practice guidelines from the Society of Obstetricians and Gynaecologists of Canada and the Canadian College of Medical Geneticists (SOGC-CCMG) [Langlois et al., 2011] support these recommendations based on a review of the literature concerning the performance of QF-PCR. The authors indicate that QF-PCR yielded conclusive results in 98.7% of the samples analyzed, and no false-positive or false-negative results for non-mosaic triploidy or trisomy 13, 18, or 21. Although no false-positive diagnoses were established for the sex chromosomes, some studies reported false-negative results; these results would be unlikely to occur at present due to the implementation of additional polymorphic markers [Langlois et al., 2011].
QF-PCR does not detect abnormalities caused by translocations, deletions, inversions, supernumerary marker chromosomes, or rare abnormalities other than trisomies 21, 18, and 13, and X and Y chromosome abnormalities [Speevak et al., 2011; Leung et al., 2008; Speevak et al., 2008; Kagan et al., 2007; Kozlowski et al., 2006; Caine et al., 2005; Ogilvie et al., 2005].

QF-PCR can identify 20% to 30% of cases of mosaicism, which is comparable to karyotyping and FISH [Baig et al., 2010, Cirigliano et al., 2009].

No false positives were obtained with QF-PCR [Langlois et al., 2011; Badenas et al., 2010; Baig et al., 2010; Cirigliano et al., 2009; Nicolini et al., 2004]. False negatives occurred more frequently prior to 2001, when the technology was still in its infancy. Thus, in a review of 13 studies with a total of 22,504 samples, Nicolini et al. [2004] reported a frequency of 9.5% for the sex chromosomes and of 0.5%, 0.9%, and 0% for autosomal trisomies 21, 18, and 13, respectively. In the most recent review by Langlois et al. [2011] of 11 studies with a total of 79,556 samples, no false negatives were reported, with the exception of two false negatives in a study that analyzed only two markers per chromosome [Kozlowski et al., 2006].

The risk of missing clinically important diagnoses ranges from 0.06% to 0.7% according to the results of 6 studies [Langlois et al., 2011; Speevak et al., 2011; Badenas et al., 2010; Comas et al., 2010; Hills et al., 2010; Ogilvie et al., 2005].
<table>
<thead>
<tr>
<th>STUDY</th>
<th>CHARACTERISTICS</th>
<th>SE % (n/N)</th>
<th>SP % (n/N)</th>
<th>PPV %</th>
<th>NPV %</th>
<th>ACCURACY %</th>
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<tbody>
<tr>
<td>Moftah et al., 2013</td>
<td>163 samples: 115 aneuploidy 13, 18, and 21 5 unbalanced rearrangements on chromosomes 18 and 21 43 controls with normal karyotype Chromosomes 13, 18, and 21</td>
<td>94.2 (113/120)</td>
<td>100 (43/43)</td>
<td>100</td>
<td>86</td>
<td>96</td>
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<td>Papoulidis et al., 2012</td>
<td>13,500 samples between 2006 and 2010 in two centres Chromosomes 13, 18, 21, X, and Y</td>
<td>75.3 (214/284)</td>
<td>99.5 (13,180/13,250)</td>
<td>75.3</td>
<td>99.5</td>
<td>99</td>
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<tr>
<td>Cirigliano et al., 2009</td>
<td>43,000 consecutive samples between 1999 and 2008 in 2 centres 37,544 AF, 4,687 CVS; 178 fetal blood; 591 fetal tissue after abortion Chromosomes 13, 18, 21, X, and Y</td>
<td>92.3 (1,608/1,741)</td>
<td>99.6 (41,019/41,178)</td>
<td>91</td>
<td>99.7</td>
<td>99.3</td>
</tr>
<tr>
<td>Speevak et al., 2011</td>
<td>4,176 samples of AF in one year Chromosomes 13, 18, 21, X, and Y</td>
<td>94.6 (331/350)</td>
<td>NA</td>
<td>100</td>
<td>NA</td>
<td>NA</td>
</tr>
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<td>Badenas et al., 2010</td>
<td>7,679 samples between 2004 and 2008 from 3 hospitals 1,243 CVS; 6,436 AF Chromosomes 13, 18, 21, X, and Y</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>98.75</td>
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<tr>
<td>Baig et al., 2010</td>
<td>1,000 samples in only one centre 978 AF; 14 CVS; 8 fetal blood Chromosomes 13, 18, 21, X, and Y</td>
<td>100 (63/63)</td>
<td>100</td>
<td>NA</td>
<td>NA</td>
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<td>Kagan et al., 2007</td>
<td>3,854 samples between 1998 and 2005 185 trisomies 13, 18, and 21; 4 mosaics; 14 abnormalities related to XY; 17 triploidy; 17 others Chromosomes 13, 18, 21, X, and Y</td>
<td>92.4 (219/237)</td>
<td>100 (3,617/3,617)</td>
<td>100</td>
<td>99.5</td>
<td>99.5</td>
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</table>

Abbreviations: AF = amniotic fluid; CVS = chorionic villus sampling; NA = not available; NPV = negative predictive value; PPV = positive predictive value; SE = sensitivity; SP = specificity; UK = United Kingdom.
5.3 Analytical (or Technical) Validity

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>PRESENCE</th>
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<tr>
<td>Repeatability</td>
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<td>Reproducibility</td>
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<td>Analytical sensitivity</td>
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<td>Analytical specificity</td>
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<td>Matrix effect</td>
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<tr>
<td>Concordance</td>
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<td>Correlation between test and comparator</td>
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<tr>
<td>Other, depending on type of test</td>
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The literature search did not identify any studies on the classic technical validation of QF-PCR used in the diagnosis of aneuploidy. However, 6 studies present results on the technique’s failures [Mann et al., 2012; Langlois et al., 2011; Speevak et al., 2011; Baig et al., 2010; Hills et al., 2010; Cirigliano et al., 2009] and report rates ranging from 0.04% and 5% for samples of amniotic fluid. Two studies [Mann et al., 2012; Hills et al., 2010] reported failure rates of 0.25% and 0.02% for chorionic villus sampling.

The failure rate in the validation study performed at the MUHC was 0.7% before the implementation of the technique and 0% after implementation. The rate of maternal cell contamination in the sample was 0.7% prior to implementation and 4.6% after implementation [Birch et al., 2013].

The concordance between the results of QF-PCR and the reference method (karyotyping) for the detection of chromosomes 21, 18, 13, and sex chromosome aneuploidy has been widely reported. First, the concordance between the two techniques can be assessed for the entire set of samples (cases of euploidy and aneuploidy). As a result of the low rate of aneuploidy and the clinical specificity of nearly 100%, the overall concordance (i.e., the clinical accuracy: true positives and true negatives for all the samples, Table 1) will be close to 100% (between 97.7% and 100%) [Papoulidis et al., 2012; Langlois et al., 2011; Speevak et al., 2011; Badenas et al., 2010; Baig et al., 2010; Lau et al., 2009; Kagan et al., 2007].

When the proportion of aneuploidy cases detected by QF-PCR is compared with that diagnosed with conventional karyotyping, the concordance is nearly 90%, especially if the targeted and clinically significant chromosomal abnormalities are considered and the limitations of the technology are taken into account. Kagan et al. [2007] found concordance of 100% for chromosome 21, 18, 13, and sex chromosome aneuploidy, but of 92.4% when the missing mosaics are included. Lau et al. [2009] reported a concordance of 88.9% with chorionic villus sampling, in which the frequency of mosaicism is well known. The concordance is 88.0% in the study by Badenas et al. [2010]. The Ontario team [Speevak et al., 2011] reported a concordance of 94.6% for all abnormalities and 98.5% for clinically significant chromosomal abnormalities that would have been detected by karyotyping. With the proposed strategy, and if karyotyping is offered in the presence of ultrasonographic signs, then the rate of concordance would be 99.2% and could reach 99.94% if the risk of abnormalities is < 4% [Speevak et al., 2011].

The performance of the Aneufast kit was compared with that of another kit, the QST-RV2 [Scott et al., 2012]. The two kits performed similarly, with identical sensitivity (97.1%) and specificity (100%) when compared with karyotyping. This would reduce the need for repeated testing (second round of reflex testing), that is, 9.7% versus 14.1% with QST-RV2, although this kit does not perform as well as the Aneufast kit in the detection of trisomy 13.
There are few quality control studies. In 2012, Susan Hamilton et al. from the NHS Foundation, in England, reported discrepancies between QF-PCR and karyotyping results obtained during two audits performed in 11 major laboratories in the United Kingdom (5 participated in the first audit and 11 in the second) and suggested simple technical modifications to improve the performance of QF-PCR [Hamilton and Waters, 2012]. In January 2012, the Association for Clinical Cytogenetics presented its best practice guidelines for the diagnosis of aneuploidy with QF-PCR [Association for Clinical Cytogenetics, 2012].

5.4 Recommendations from Other Organizations

In its 2011–2014 Model of Best Practice for prenatal screening for Down syndrome, the National Health Service (NHS) in the United Kingdom recommends QF-PCR as a diagnostic test to confirm fetal abnormalities [NHS England, 2013].

The use of QF-PCR for the systematic screening of aneuploidy was established in Sweden in 2005 [Badenas et al., 2010] and in the United Kingdom in the early 2000s [Mann et al., 2012]. In 2008, the Society of Obstetricians and Gynaecologists of Canada (SOGC) was already suggesting the use of this technique [Sparkes et al., 2008], and firm recommendations for the systematic use of the test, in conjunction with appropriate prenatal genetic counselling, were implemented in 2011 [Langlois et al., 2011].

6 ANTICIPATED OUTCOMES OF INTRODUCING THE TEST

6.1 Impact on Material and Human Resources

Ogilvie et al. [2005] presented the results of a technology assessment report published in 2003 in the United Kingdom, finding that 4 individuals (professionals and technicians) were needed to perform 12,000 QF-PCR-based tests, compared with 30 people for the same number of karyotype tests.

No tests were carried out in Quebec in preparation for this notice.

6.2 Economic Consequences of Introducing Test Into Quebec's Health Care and Social Services System

The calculations are based on the figures provided by the requestor:
- diagnosis of very high-risk cases: $53,400 (300 tests $178 per test) per year;
- diagnosis of high-risk cases for RUIS McGill only: between $142,400 and $160,200 per year (between 800 and 900 tests $178 per test);
- diagnosis of high-risk cases for the province: between $534,000 and $623,000 per year ($178 per test between 3,000 and 3,500 cases);
- Other cases would be added if the test were used for postnatal diagnosis.

6.3 Main Organizational, Ethical, and Other (Social, Legal, Political) Issues

Time plays a crucial role in prenatal diagnosis, as it allows pregnant women to benefit from adequate genetic counselling and receive reassurance or help in making difficult decisions concerning the continuation of the pregnancy. Therefore, it is essential for them to have access to rapid and accurate tests [Langlois et al., 2011; Dickinson et al., 2009; Conrad et al., 2005].

Excessive workloads, as well as the number of people needed to perform the test in genetic laboratories, are another factor to consider. According to the literature, QF-PCR requires less time and fewer personnel than karyotyping [Sparkes et al., 2008; Conrad et al., 2005; Ogilvie et al., 2005b].
If QF-PCR is offered as a first-line test for pregnant women who have received a result indicating a high risk (abnormal ultrasound, abnormal prenatal screening results, advanced maternal age, or family history of chromosomal abnormalities), in the case of positive results, karyotyping is performed to confirm the diagnosis. The rate of karyotype testing is estimated at 13% [Speevak et al., 2011] to 16% [Kagan et al., 2007] and 22% [Badenas et al., 2010], with a detection rate of chromosomal abnormalities ranging from 99.92% to 98% and 99.95%, respectively.

Prenatal screening, particularly for trisomy 21, has been the source of many debates in the community and ultimately involves the pregnant woman and her partner. Prenatal genetic information must be complete, impartial, and understandable. The choice to undergo routine screening must be based on free and informed decision-making. The SOGC [Langlois et al., 2011] emphasizes the importance of providing genetic counselling, particularly for this technique, as well as training for counsellors on the advantages and limitations of the test at the start of the systematic implementation of QF-PCR.

Counselling as well as screening must be accessible and provided at the appropriate stage of the pregnancy. When a diagnosis of chromosomal abnormality is made, a specialized (and at times multidisciplinary) team should be available to enable the mother or the couple to make a free and informed choice concerning the continuation of the pregnancy. When the test is performed after the birth, a multidisciplinary approach is often taken, with full respect for the parents’ autonomy and values.

7 IN BRIEF

7.1 Clinical Relevance

QF-PCR is a well-established molecular genetic technique that is rapid, robust, accurate, and cost-effective for the detection of the most common aneuploidies during pregnancy. One of its main advantages is that it provides results within 24 to 48 hours, unlike karyotyping, which requires 2 to 3 weeks. This would considerably reduce parents’ anxiety and enable them to make any decision needed at a much earlier stage of pregnancy. However, this test involves an invasive procedure—amniocentesis or chorionic villus sampling—which carries a risk of complications that may lead to fetal loss (1/200 to 1/400 with amniocentesis in the second trimester and up to 3% to 5% with chorionic villus sampling during the first trimester).

7.2 Clinical Validity

This test has a sensitivity of > 90%, a specificity of 100%, and an accuracy of nearly 100%. There are virtually no false positives or false negatives. This test can detect only 20% to 30% of mosaicisms, as does FISH. The risk of missing a clinically important diagnosis ranges from 0.06% to 0.7%, depending on the study referred to.

7.3 Analytical Validity

The technique’s failure rate is 0.25% to 0.02%, depending on the study referred to, and is mainly attributable to the contamination of the sample by maternal cells. The overall concordance with the reference test is nearly 100%. The concordance is greater than 90% when pathological cases identified by QF-PCR are compared with karyotyping, but it is 98% and 99% for clinically significant chromosomal abnormalities. Quality control has been implemented in the United Kingdom [Hamilton et al., 2012] by the Association for Clinical Cytogenetics and the Clinical Molecular Genetics Society [2012], but few studies are available on the subject.
7.4 Recommendations from Other Organizations

This technique has been used to screen for chromosome 21, 18, 13, and sex chromosomes aneuploidy since the early 2000s in the United Kingdom and since 2005 in Sweden and in other European countries. The SOGC proposed it in 2008 and recommended it in 2011.
8 INESSS NOTICE IN BRIEF

Detecting aneuploidy of chromosomes 13, 18, 21, X, and Y with QF-PCR

<table>
<thead>
<tr>
<th>Status of the Diagnostic Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>☒ Established</td>
</tr>
<tr>
<td>☐ Innovative</td>
</tr>
<tr>
<td>☐ Experimental (for research purposes only)</td>
</tr>
<tr>
<td>☐ Replacement for technology: ____________, which becomes obsolete</td>
</tr>
</tbody>
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<thead>
<tr>
<th>INESSS Recommendation</th>
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<tbody>
<tr>
<td>☒ Include test in the Index</td>
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<tr>
<td>☐ Do not include test in the Index</td>
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<tr>
<td>☐ Reassess test</td>
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</tbody>
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<tr>
<th>Additional Recommendation</th>
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<tbody>
<tr>
<td>☐ Draw connection with listing of drugs, if companion test</td>
</tr>
<tr>
<td>☐ Produce an optimal use manual</td>
</tr>
<tr>
<td>☐ Identify indicators, when monitoring is required</td>
</tr>
</tbody>
</table>

**NOTE**
Well-established test with significant anticipated benefits.

**NOTE to MSSS**
Ensure a reduction in the number of karyotype tests performed as well as the number of hours devoted to this technique in cytogenetics laboratories.
REFERENCES


Hamilton SJ and Waters JJ. Completely discrepant results between prenatal QF-PCR rapid aneuploidy testing and cultured cell karyotyping obtained from CVS: Lessons from UK audit and re-audit of 22,221 cases. Prenat Diagn 2012;32(9):909-11.


