

Cell-free fetal nucleic acids for non-invasive prenatal diagnosis

Report of the UK expert working group



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Disclaimer: the field of non-invasive prenatal diagnosis is extremely dynamic and technology is developing very rapidly; this report is accurate as of 7th January 2009.

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Executive Summary

This report reviews the scientific and clinical status of the use of circulating cell-free fetal nucleic acid (cffNA) technology for non-invasive prenatal diagnosis (NIPD), a rapidly developing and dynamic field. It also examines some of the major ethical, social and legal implications of the technology, and highlights some of the key issues that will need to be addressed if cffNA testing is to be implemented for different applications within the NHS. The report has been produced by a Working Group consisting of representative stakeholders, including relevant clinicians (GPs, obstetricians, midwives and geneticists), scientists, NHS commissioners, public health experts, ethicists and patient representatives.

The landmark discovery of cell-free fetal DNA (cffDNA) in maternal blood during pregnancy was made more than a decade ago. At the time it was recognised that, although cffDNA represents only a small fraction of the total cell-free DNA in the maternal circulation during pregnancy, the fact that it can be reliably detected from 7 weeks gestation, and is comprehensively cleared within a few hours of birth, could have important clinical consequences. The promise of that breakthrough is now being realised as the technology is translated into clinical practice for the non-invasive prenatal detection of specific fetal genetic traits.

In this report, we identify four distinct clinical applications of the technology: first, fetal sex determination in pregnancies at risk of a sex-linked disease, through detection of male Y chromosome DNA; second, diagnosis of certain single gene disorders, particularly through detection of a paternally inherited mutation; third, detection of fetal aneuploidy such as Down's Syndrome, in which there is a small but detectable change in the ratio between chromosomes; and fourth, diagnosis of fetal blood type in pregnancies at risk of incompatibility, particularly RhD. Although technical development is still ongoing, the diagnostic accuracy of the technique is expected to be very high in all applications.

To date, the translation of cffNA technology from research into clinical practice for each application has been somewhat fragmented, and influenced by the supporting infrastructure, relevant service provision and clinical setting. This report seeks to synthesise these different applications in order to provide a service-based overview of the implications for the NHS of implementing this technology. Hence, the potential applications have been divided into three categories in terms of their implications for antenatal care: specialist genetic services for high risk families, standard antenatal testing, and routine management of pregnancy. In the first two categories, cffNA testing could significantly reduce the use of invasive techniques for prenatal diagnosis (such as amniocentesis and chorionic villus sampling, which currently result in miscarriage in around 1% of cases), as well as allowing earlier prenatal diagnosis; the technology is also likely to be substantially cheaper than current invasive diagnostic testing. In the final category, testing could improve patient care through better targeting of anti-D therapy, which is currently given to all RhD negative women without testing to prevent a potentially fatal maternal immune response against a RhD positive fetus.

This service-based perspective raises a number of wider issues. Firstly, it highlights the ethical challenges to implementing cffNA testing within specific NHS services, including safeguarding patient autonomy, providing for informed consent, ensuring equity of access and avoiding adoption of the technology in new clinical areas without sufficient clinical consideration and ethical justification (a phenomenon known as specification creep). Secondly, in reviewing the implications of cffNA testing as a whole, it becomes apparent that there are a number of non-clinical applications of the technology, such as social sex selection and paternity testing. These tests are already available directly to the consumer over the internet, raising questions as to whether policy should be developed to regulate direct public access to these tests.

In the UK, tests based on the analysis of cffDNA have been available as a service to hospital trusts since 2001 to determine fetal RhD status in high risk sensitised women, and is also available in some NHS hospitals for fetal sex determination where there is clinical justification. In the US, the technology is currently being developed and tested by the diagnostics company Sequenom[®], which expects to launch a commercial screening test for Down's Syndrome during 2009. Nonetheless, there are a number of key issues that need to be addressed before this technology can be widely implemented in the NHS.

The working group believes that the implementation of non-invasive prenatal diagnosis for clinically significant genetic disorders is desirable, both to improve the quality and management of antenatal care and to facilitate parental reproductive choice, and that the development of cell-free fetal nucleic acid technology for these purposes should be supported within the UK. The key findings and recommendations from the working group are as follows:

- (1) NIPD using cell-free fetal DNA (and RNA) is likely to become increasingly available within the next 3-5 years, and the NHS should take steps now to ensure that it is able to respond in a timely and appropriate manner as the technology develops.
- (2) Public engagement is urgently needed and should be pro-actively pursued with a clear, accurate and consistent message that recognises the limitations of cffNA testing.
- (3) Professional education is urgently needed, particularly for key health care workers, and should be started immediately by relevant professional bodies.
- (4) Formal evaluation of the use of cffNA testing for each application within a specified care pathway should be undertaken prior to implementation within the NHS.
- (5) Formal audit and monitoring processes should be established for all non-invasive prenatal tests based on cffNA technology.
- (6) National best practice guidelines should be developed by key clinical services to ensure that cffNA testing is only used within agreed clinical pathways.
- (7) Standard protocols should be developed by expert laboratory services that include the whole care pathway, supplemented by quality assurance frameworks to ensure accuracy, reliability and comparability of results.
- (8) Private NIPD services are already available on a direct-to-consumer basis, which will impact on NHS services; although the extent to which regulation can and should be applied to these services is debatable, development of a voluntary code of conduct should be supported to help ensure quality.
- (9) Oversight from appropriate authorities is needed to ensure responsible, effective and timely implementation of cffNA technology for NIPD within the NHS.
- (10) Additional research is needed to investigate some of the broader implications of cffNA testing for NIPD.

Working Group Representation

The following professional bodies and advisory groups were represented on the working group; a full list of the members of the working group is given in Appendix I.

Patients

- Antenatal Results & Choices
- Genetic Interest Group

Physicians and scientists

- Antenatal & Child Health Screening Programme
- British Society of Fetal & Maternal Medicine
- British Society of Human Genetics
- European SAFE Network of Excellence
- Joint Committee on Medical Genetics
- National Blood Service
- NHS Sickle Cell & Thalassaemia Screening Programme
- NHS Fetal Anomaly Screening Programme
- Royal College of General Practitioners
- Royal College of Midwives
- Royal College of Obstetricians & Gynaecologists
- Royal College of Pathologists
- Royal College Physicians
- UK National Screening Committee

Laboratories

- Great Ormond Street Hospital Regional Molecular Genetics Laboratory
- International Blood Group Reference Laboratory
- Manchester National Genetics Reference Laboratory
- National Haemoglobinopathy Reference Laboratory
- Wessex National Genetics Reference Laboratory

Commissioners and NHS Advisory bodies

- Human Genetics Commission
- UK Department of Health
- UK Genetic Testing Network

Steering Group

Dr Hilary Burton	Public Health Consultant, Programme Director, PHG Foundation, Cambridge
Dr Lyn Chitty	Reader in Clinical Genetics and Fetal Medicine, UCL Institute of Child Health
Dr Tessa Homfray	Consultant Clinical Geneticist, St Georges Hospital Medical School
Dr Ainsley Newson	Senior Lecturer in Biomedical Ethics, Centre for Ethics in Medicine, University of Bristol
Mrs Gail Norbury	Director, NE Thames Molecular Genetics Laboratory, Great Ormond Street Hospital
Professor Peter Soothill	Head of the Division of Obstetrics and Gynaecology, University of Bristol

Glossary of Acronyms

ACCE	Analytical validity, Clinical validity, Clinical utility, ELSI
AFP	Alpha fetoprotein
β-hGC	Free β-human chorionic gonadotropin
cffDNA	cell-free fetal DNA
cffNA	cell-free fetal Nucleic Acids
cffRNA	cell-free fetal RNA
CVS	Chorionic Villus Sampling
DS	Down's Syndrome (trisomy 21)
DTC	Direct-To-Consumer
ELSI	Ethical, Legal and Social Issues
HDN	Haemolytic Disease of the Newborn
HFEA	Human Fertilisation and Embryology Authority
HGC	Human Genetics Commission
IP	Intellectual Property
IVDD	<i>In Vitro</i> Diagnostics Directive
IVF	<i>In Vitro</i> Fertilisation
JCMG	Joint Committee on Medical Genetics
MHRA	Medicine and Healthcare products Regulatory Agency
NHSC	NHS National Horizon Scanning Centre
NICE	National Institute of Clinical Excellence
NIHR	National Institute of Health Research
NIPD	Non-Invasive Prenatal Diagnosis
NSC	National Screening Committee
NT	Nuchal translucency
PAPP-A	Pregnancy associated plasma protein A
PCR	Polymerase Chain Reaction
QF-PCR	Quantitative fluorescent ('real-time') PCR
RAPID	Reliable Accurate Prenatal non-Invasive Diagnosis
RCM	Royal College of Midwives
RCOG	Royal College of Obstetricians and Gynaecologists
RhD	RhD blood antigen, commonly known as Rhesus (Rh) factor D
mRNA	Messenger RNA
SAFE	Special Non-Invasive Advances in Fetal and Neonatal Evaluation
SNP	Single Nucleotide Polymorphism
SRY	Sex-determining Region Y
STR	Short Tandem Repeats
uE₃	Unconjugated oestriol
UKGTN	UK Genetic Testing Network

Glossary of Terms

Accuracy	Proportion of patients for whom the correct diagnosis has been made, <i>i.e.</i> proportion of both true positive and true negative tests
Allele	Variant forms of the same gene
Amniocentesis	Invasive procedure used for prenatal diagnosis in which a sample of amniotic fluid is removed and examined
Aneuploidy	Deviation in the number of chromosomes per cell from the normal 46
Antibody	Protein produced by the immune system in response to a foreign substance (see <i>antigen</i>)
Antigen	Any foreign substance, often a cell surface protein, that stimulates the immune system to make a specific antibody in response (see <i>antibody</i>)
Autosomes	Chromosomes other than the sex chromosomes
Base-pair	Pair of complementary nucleotides that make up the DNA sequence
Chromosome	Single, long molecule of DNA within cells that carries genetic information; humans have 22 pairs of <i>autosomes</i> plus one pair of sex chromosomes (XX in women, and XY in men); one member of each pair of chromosomes is inherited from the father, and the other from the mother
CVS	Chorionic villus sampling; invasive procedure used for prenatal diagnosis in which a sample of the placenta is removed and examined
DNA methylation	Chemical modification of the DNA molecule which does not change the genetic sequence but results in heritable gene silencing
Dominant inheritance	Inheritance of a single copy of a mutation from one parent only (or arising <i>de novo</i> during egg or sperm formation), which is sufficient to cause disease
Epigenetic	Heritable influence on gene activity that does not involve changes to the DNA sequence itself
Genotype	Specific genetic constitution of an individual
Heterozygous	Two different alleles, one on each of a pair of chromosomes, at a particular position in the genome of an individual
Homozygous	Two identical alleles, one on each of a pair of chromosomes, at a particular position in the genome of an individual
Karyotype	Description of the number and structure of all the chromosomes of an individual
Mosaicism	Phenomenon in which genetically different tissues occur in the same organism, including cells with a different number of chromosomes
Mutation	Relatively rare change in the DNA sequence from the normal sequence
Non-coding	Region of the DNA sequence which does not contain any genes, <i>i.e.</i> does not code for the production of a functional protein; around 99% of the human genetic code is currently thought to be non-coding
Nucleic acids	Molecule comprising a sequence of nucleotides, including DNA and RNA

Nucleotide	Molecular unit from which DNA is made, consisting of adenine (A), guanine (G), cytosine (C) or thymine (T) ordered in a specific sequence
PCR	Polymerase chain reaction; molecular biology technique in which a fragment of DNA with a specific sequence is copied or amplified exponentially
Phenotype	The observable traits of an organism
Plasma	Non-cellular liquid component of blood
Point mutation	A DNA sequence variation that involves substitution, insertion or deletion of a single base A, C, G or T (also see <i>SNP</i>)
Polymorphism	Common variation in a region of DNA sequence
Promoter	DNA sequence that regulates the activity of a gene
QF-PCR	Quantitative fluorescent <i>PCR</i> ; molecular biology technique in which the products of a <i>PCR</i> reaction are monitored in real-time
Recessive inheritance	Inheritance of a mutation from both parents (two copies) is required to cause disease; parents are usually unaffected carriers
Sensitivity	Proportion of those with a condition who have a positive test result (inversely related to the proportion of false negative results)
Serum	Blood plasma with clotting proteins removed
Silencing	Preventing the genetic code for a gene from being translated into a functional protein
SNP	Single nucleotide polymorphism; common variation in a single base at a particular position in the genome
Specificity	Proportion of those without a condition who have a negative test result (inversely related to the proportion of false positive results)
Trimester	One of three time periods during pregnancy, each of which is approximately 3 months
Trisomy	A form of aneuploidy in which there is an additional (third) copy of a particular chromosome per cell

1 Introduction

The use of cell-free fetal nucleic acids (cffNA) for non-invasive prenatal diagnosis (NIPD) was discussed at a meeting of the Joint Committee on Medical Genetics (JCMG) in 2007, and it was suggested that a strategy for implementing this technique in clinical practice across the UK was needed. Tessa Homfray and other members of the JCMG agreed to lead a small working party, which met once and reported back to the JCMG in October 2007. Members of the working party decided that the involvement of a wider group of stakeholders was needed in order to form recommendations about the key issues in implementation.

The PHG Foundation (an organisation dedicated to the application and evaluation of genome-based technologies for the benefit of health) volunteered to set up a working group, consisting of a steering committee and a wider group of stakeholders, and run two workshops that would explore the issues further. The workshops were held on 6th May and 30th September 2008. A full list of the working group members is given in Appendix I. In addition, the PHG Foundation produced background materials for the workshops, including a technical background paper, which formed the basis for a subsequent peer-reviewed publication (abstract in Appendix II), and a review of the ethical, legal and social issues, produced in collaboration with lecturers from the Hughes Hall Centre for Biomedical Science in Society, University of Cambridge (abstract in Appendix III).

Although cffNA testing is currently already being used in some expert centres for selected clinical applications, it does not yet form part of the recognised standard care offered by specialist genetics, fetal medicine and antenatal services that is available to all through the NHS. The purpose of the working group was therefore to review the use of cell-free fetal nucleic acid technology for different prenatal applications within UK clinical services, in order to make recommendations towards the development of an appropriate strategy for implementation throughout the NHS.

The primary objectives of the working group were identified as:

- (1) To review the scientific literature in addition to the ethical, legal and social implications (ELSI) and current service provision;
- (2) Summarise unresolved issues and identify outstanding research needs;
- (3) Make recommendations for strategies necessary for timely implementation of the technology into standard practice and disseminate these findings.

Several research networks and collaborations already exist in this area, both in the UK and Europe. The largest of note is the European *Special non-invasive Advances in Fetal and neonatal Evaluation (SAFE) Network of Excellence* (www.safenoe.org) established in March 2004, funded under the EU Framework 6 programme with 12 million Euros over a period of 5 years. It currently comprises 49 partners from 19 countries. The network was originally set up following recognition that relevant knowledge is dispersed across many disciplines including molecular biology, medical genetics, bioinformatics, social science, and ethical studies; its aim was to bring together leading experts from all the key disciplines in a programme designed to achieve intellectual and practical integration. Its overall goal was to enhance the efficacy of non-invasive prenatal diagnosis (and neonatal screening) for genetic disorders within and beyond the European Community.

In October 2008, the UK National Institute for Health Research (NIHR) awarded a £2 million grant to a study entitled Reliable Accurate Prenatal non-Invasive Diagnosis (RAPID), which will run for 5 years from April 2009 led by Dr Lyn Chitty. This integrated project includes technology development, clinical evaluation, economic modelling and patient consultation, with the objective of clinical implementation of non-invasive prenatal diagnosis in the NHS. In addition, it has also awarded a smaller grant specifically to look at implementing early RhD testing within routine antenatal care, including performing a full economic analysis.

A review of the area and the findings of the working group are presented in this report, including specific policy recommendations for the effective and appropriate implementation of NIPD using cffNA within the NHS. This report aims not only to provide the Joint Committee and Department of Health with a timely summary of recent developments and implications of cffNA technology for NIPD, but also to act as a link from the completion of the SAFE network funding to the beginning of the RAPID project. Appropriately, therefore, many of the UK-based members of the SAFE network, and most of the recipients of the relevant NIHR grants are also members of the working group involved in producing this report.

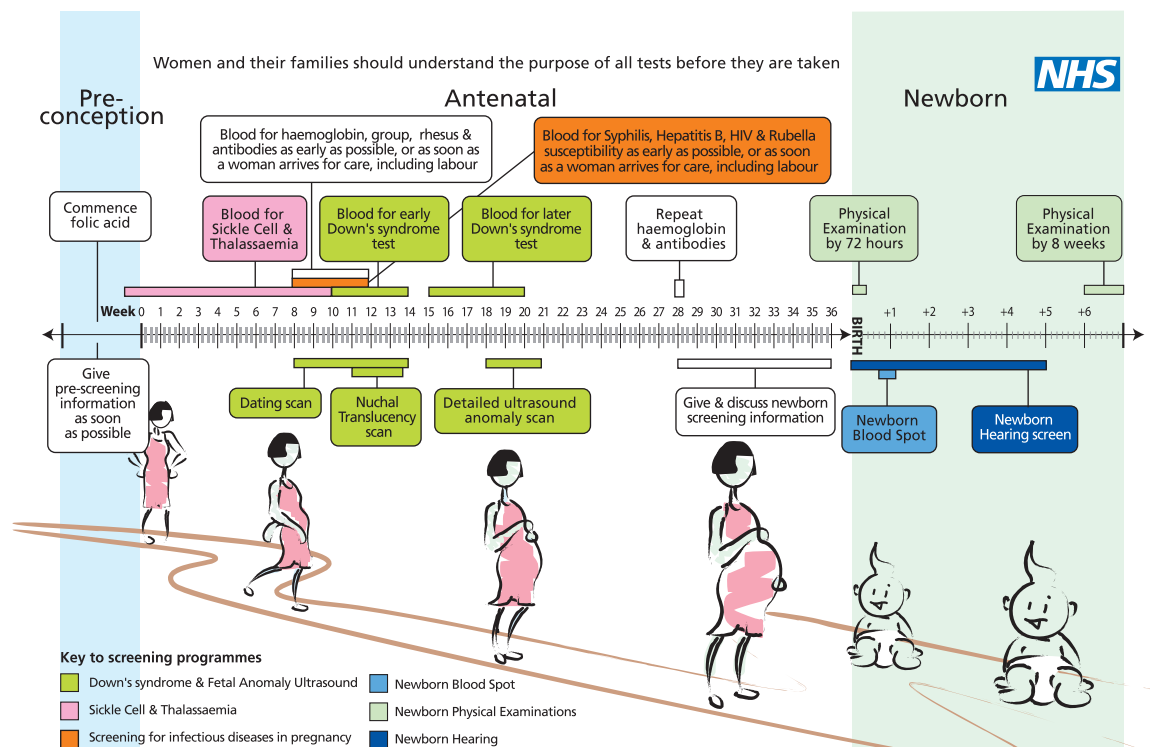
2 Background to prenatal testing

Prenatal screening and diagnosis are routinely offered in antenatal care, and are considered to be important in managing pregnancy and allowing women to make informed choices about the continuation of pregnancies affected by developmental abnormalities. Prenatal testing forms part of established obstetric practice in many countries, and genetic and chromosomal abnormalities account for around 1% of terminations in the UK.

Prenatal testing falls into two categories. Firstly, prenatal *screening* is offered to all pregnant women as part of routine antenatal care to determine if their fetus is at significant *risk* of having a particular disorder, such as Down's Syndrome or sickle cell anaemia. Secondly, in cases deemed to be high risk, prenatal *diagnosis* is offered, which aims to accurately determine whether the fetus has a particular disorder or characteristic.

Routine antenatal care in the UK is a structured pathway, which comprises numerous visits to a GP or midwife at specific times during the pregnancy (see Figure 2-1 for a summary; National Screening Committee 2008). It includes a number of screening tests to determine if the pregnancy is at high risk of a particular genetic abnormality, including blood tests, family history, physical examination and ultrasound. Screening tests for specific genetic disorders include determining carrier status for common recessive genetic disorders, such as sickle cell anaemia, and risk of Down's Syndrome.

Figure 2-1 Timing of tests in the routine NHS antenatal care pathway in England
Used with permission from the UK National Screening Committee Antenatal and Newborn Programmes, England (2008)



www.screening.nhs.uk/an
Screening Timeline Version 2, March 2008

Screening Timeline - optimum times for testing

Typically, a woman will first visit her GP if she suspects she is pregnant, who will confirm the pregnancy and expected delivery date, determine any medical problems or history, give information about all antenatal screening tests, and make the referral to the obstetric unit. In an uncomplicated pregnancy, a midwife will organise regular antenatal appointments, during which various tests are offered, including:

- **≤10 weeks - booking appointment.** Numerous tests discussed including screening tests (e.g. infections, Down's Syndrome), blood group analysis and dating scan; family history taken to identify high risk pregnancies. Note that the exact date for this appointment varies, as it depends upon when the woman first identifies herself as being pregnant;
- **16-18 weeks** - review results of all screening tests;
- **18-20 weeks** - fetal anomaly ultrasound scan;
- **28 weeks** - screening for atypical blood cell antibodies. First dose of anti-D offered to RhD negative women;
- **34 weeks** - second dose of anti-D offered to RhD negative women.

Women identified as being at high risk of having a pregnancy affected by a genetic or chromosomal abnormality, either as a result of screening or family history, are rapidly referred to an obstetrician or directly to a clinical geneticist and may be offered diagnostic testing. This involves taking a sample of fetal cells from inside the uterus for genetic analysis, using one of the following methods:

- (1) **Chorionic villus sampling (CVS)** - a small sample of the placenta is taken for testing, either by inserting a needle through the abdominal wall or by passing a fine tube or forceps through the cervix; in around 8% of women, not enough tissue can be removed and the needle must be reinserted. This procedure is usually carried out between 11-14 weeks gestation. CVS causes miscarriage in around 1-2% of cases [Mujezinovic & Alfirevic 2007].
- (2) **Amniocentesis** - a small amount of the amniotic fluid surrounding the fetus in the womb is removed for testing by passing a needle through the abdominal wall; in around 2% of women, not enough fluid can be removed and the needle must be reinserted. This procedure is usually carried out after 15 weeks gestation. Amniocentesis causes miscarriage in around 1% of cases [Mujezinovic & Alfirevic 2007].

Although the subsequent laboratory assays are nearly 100% accurate, the relatively high occurrence of multiple needle insertions means that clinical sample failure is not uncommon. Both the techniques also carry a small risk of miscarriage, and although CVS carries a higher risk, there are substantial advantages to earlier diagnosis. If a decision is taken to terminate the pregnancy, early termination is medically induced or involves surgical vacuum aspiration, whilst termination at more than 14 weeks through the NHS may require inducing labour. Early diagnosis also gives women longer to reflect and consider their options thoroughly.

The invasive approach to obtaining fetal tissue and DNA is currently the gold standard for prenatal diagnosis. However, many women are reluctant to undergo invasive testing, either due to the small but significant risk to the pregnancy, or because they would not terminate the pregnancy irrespective of the results. Of the approximately 700,000 pregnant women *per annum* in the UK, around 20,000 amniocentesis tests and 5,200 CVS tests are conducted annually (Association of Clinical Cytogenetics audit 2006-07), with an estimated pregnancy loss of around 250 due to miscarriage.

A reliable and convenient method for non-invasive prenatal diagnosis (NIPD) has therefore long been sought to reduce this risk of miscarriage and allow earlier diagnosis. Despite extensive academic research in this field, thorough clinical evaluation is still needed before a new test can be used in practice, to ensure that it offers value to society. This is dependent upon its diagnostic usefulness, benefit to patients, cost-effectiveness and feasibility in clinical practice. Proper test evaluation is critical to providing excellence of care, ensuring patient safety and setting health care priorities. Recent progress in the technological development of NIPD means that tests are now nearing clinical practice, making evaluation and review appropriate at this time.

Although some work has investigated using fetal cells obtained from urine or cervical mucus, most research has focused on strategies for detecting genetic elements from the fetus present in the maternal blood.

3 Cell-free fetal nucleic acids

Contrary to popular belief that the placenta forms an impermeable barrier between mother and child, there is bidirectional traffic between the fetus and mother during pregnancy. Multiple studies have shown that both intact fetal cells and cell-free fetal nucleic acids (cffNA, i.e. DNA and RNA) cross the placenta and circulate in the maternal blood stream. There are numerous issues relating to the use of cffNA for NIPD, including technical and clinical as well as ethical, social and legal, which require detailed consideration. General information is summarised in this chapter, and detailed issues relating to specific applications are presented in each of the relevant chapters; details of more comprehensive and fully referenced reviews can be found in Appendix II and III.

Cell-free fetal DNA (cffDNA) from the Y chromosome of male fetuses was first discovered in maternal blood by Dennis Lo, then at the University of Oxford and now Professor of Medicine at the Chinese University of Hong Kong, and reported in a seminal paper published in the *Lancet* in 1997 [Lo *et al.* 1997]. This DNA originates from placental cells, which expel their DNA into the maternal circulation during normal cell death, breaking up the chromosomes into short fragments. In addition, cell-free fetal RNA (cffRNA) derived from placentally active genes was also detected in the maternal circulation in 2000 [Poon *et al.* 2000], and found to have similar properties to cffDNA (see Table 3-1 for a summary).

Unlike fetal cells, which can persist for decades, cffNA are rapidly cleared from the maternal circulation and are undetectable 2 hours after delivery [see Lo & Chiu 2007 for review]. Although all fetal elements in the maternal blood are necessarily diluted by maternal elements, the amount of cffDNA increases with gestation and comprises around 5-10% of the total cell-free DNA in maternal blood during the first and second trimesters [Lun *et al.* 2008a]. The exact amount and proportion of cffDNA varies between individuals, but it can be reliably detected from around the 7th week of gestation.

Table 3-1 Summary of key properties of fetal/placental elements in maternal blood, specifically cell-free fetal nucleic acids and intact fetal cells.

Properties	cffNA	Fetal Cells
Earliest detection	4 weeks	~ 7 weeks
Proportion	5-10%	0.0001 - 0.01%
Persistence in maternal blood	< 24 hours	> 27 years
Relevant physical properties	Short fragments	Dense nucleus

3.1 Technical overview

Distinguishing, or ideally isolating, fetally derived cell-free DNA in an overwhelming background of maternal cell-free DNA is a significant technical challenge for three principal reasons. First, the concentration of all cell-free DNA in blood is relatively low; second, cell-free *fetal* DNA molecules are substantially outnumbered by cell-free *maternal* DNA molecules; and third, the fetus inherits half its genetic sequence from the mother, making much of the cffDNA indistinguishable from maternal cell-free DNA.

A number of methods have been developed to address these problems. During isolation of the cell-free fraction of maternal blood followed by DNA extraction, it is critical to ensure that no cells are present and that the maximum possible amount of DNA is extracted. Following DNA isolation, small differences between the fetal and maternal DNA sequences are exploited in order to specifically detect only fetal DNA. Since the proportion of fetal DNA is relatively small, a highly sensitive technique must be used to detect fetal-specific DNA sequences. Most commonly, polymerase chain reaction (PCR) is used, which works by exponentially copying a specific sequence of DNA, thus vastly increasing the proportion of DNA with that specific sequence relative to other sequences. This process can be monitored in real-time (through quantitative fluorescent QF-PCR) using fluorescent probes that bind to the DNA sequences of interest, which allows quantification of the amount of specific DNA present in the sample. More recently, mass spectrometry has also been applied to the identification of cffDNA, in which the precise mass of each DNA fragment is analysed to determine the sequence and hence detect fetal-specific alleles.

As with all tests, the accuracy and reliability of detection can be significantly improved by increasing the signal-to-noise ratio. Selective enrichment of the fetal portion of cell-free DNA is possible because there is a difference in the average physical length of fetal and maternal DNA. Fetally-derived DNA fragments are generally smaller than those that are maternally-derived; nearly all fetal cell-free DNA, but only just over half of maternal cell-free DNA, is less than 300 base-pairs in length. Therefore, size fractionation can be used to increase the proportion of fetal DNA, which may increase the sensitivity of detection.

A major area of current research is to find universal fetal specific markers, which can be used either as diagnostic tests in their own right, or to confirm and quantify the presence of fetal DNA independent of sex or other specific diagnostic tests. These markers could be used alongside clinically relevant diagnostic tests as a positive control for the presence of cffDNA, in order to highlight false negative results caused by problems with either the DNA extraction process or levels of circulating DNA below the detection limit.

One method under investigation is the detection of specific DNA sequences located on the autosomal chromosomes that can be shown to be paternally inherited, including single nucleotide polymorphisms (SNPs) and short tandem repeat sequences (STRs), where the number of repeats differs between the maternal and paternal genomes. However, due to issues with paternity and similarity of maternal and paternal SNPs, the ideal fetal marker would be entirely independent of paternally inherited sequences, and instead be truly universal to all fetuses. To date, two methods have been proposed that are able to fulfil all the requirements for a universal fetal marker, based on exploiting differences in the activity of certain genes (such as those required for growth and development) between the fetus or placenta and the mother:

- (1) Detection of epigenetic differences between the mother and fetus (specifically DNA methylation), which result in different levels of activity in certain genes between cells of the mother versus the growing fetus. For example, the promoter region of two tumour suppressor genes - *maspin* and *RASSF1A* - are differentially methylated in the placenta relative to maternal cells [Lo 2006].
- (2) Detection of mRNA which is uniquely derived from active fetal DNA. Numerous RNA species have been shown to be of placental origin and detectable in maternal plasma during pregnancy. Since the expression of these genes is unique to pregnancy, complete isolation from background maternal RNA is possible [Ng *et al.* 2003].

3.2 Clinical applications

There are a number of quite discrete clinical applications of cffNA analysis for NIPD, based on distinct and detectable differences between fetal and maternal genomes. These applications fall into three categories of clinical antenatal care:

- (1) **High genetic risk families** (see *Chapter 4*) where there is a family history of a known heritable genetic disorder. In this case, cffNA testing would essentially be a replacement technology for invasive diagnostic testing, with a few specific applications:
 - i. fetal sex determination (in the case of sex-linked disorders and certain endocrine disorders);
 - ii. certain autosomal single gene disorders (particularly where the paternal disease causing allele differs from the maternal one).
- (2) **Routine antenatal screening** (see *Chapter 5*) for all pregnancies at a population level. Rather than being essentially a replacement technology, cffNA has the potential to change the nature of these tests. Specific applications include testing for Down's Syndrome and other aneuploidies.
- (3) **Management of pregnancy** (see *Chapter 6*) for pregnancies at risk of specific complications. This includes blood group incompatibilities, such as fetal RHD testing in RhD negative women (who currently receive anti-D prophylaxis without testing).
Note that this report specifically excludes management of other pregnancy complications¹ that do not aim to determine the fetal genotype.

These applications are all at different stages of technical development and clinical application. In the UK, determination of fetal RhD status is now accepted practice for management of high risk sensitised pregnancies, and fetal sex determination is also becoming increasingly commonplace in specialist centres for pregnancies at risk of specific inherited diseases.

There are still a number of technical and clinical obstacles to achieving high diagnostic accuracy in any of these applications, the majority of which are expected to be overcome through ongoing research. The potential for false results is common to all the applications: false negatives can be the result of failure to extract or detect sufficient material, due to the low amount of total cell-free DNA and the small proportion of fetal versus maternal cell-free DNA; false positives can be the result of either technical issues, such as contamination, or clinical abnormalities such as the presence of a non-identical vanishing twin. Further evaluation is therefore needed to determine the clinical sensitivities and specificities of each application.

3.3 Alternative technologies

There are several competing technologies that could also potentially be used for NIPD using a maternal blood sample. In particular, intact fetal cells circulating in maternal blood present an attractive target for NIPD [Purwosunu *et al.* 2006], particularly for the diagnosis of fetal sex and chromosomal abnormalities by simply analysing the number and type of chromosomes present (karyotyping). The existence of fetal cells in maternal blood has been known for more than a century, but isolation of intact fetal nucleated red blood cells for the purpose of prenatal diagnosis was not achieved until 1990 [Bianchi *et al.* 1990]. Since then, the isolation and detection of numerous different types of fetal cells from maternal blood has been extensively investigated, and various methods of fetal cell enrichment developed, with varying degrees of success. Most results to date have been disappointing, due to the scarcity of intact fetal cells in the maternal circulation, a low efficiency of enrichment,

¹ Elevated levels of cffDNA are often observed in pre-eclampsia, abnormal placentation and preterm labour, and may therefore have utility as a biomarker for early diagnosis and pregnancy management.

lengthy persistence of fetal cells after pregnancy, and problems with chromosomal analysis caused by an abnormally dense nucleus.

The other main competitor to cffNA for NIPD from a maternal blood sample is proteomics, in which either the presence, quantity or particular combination of specific fetal or maternal proteins is measured in order to screen for or diagnose a particular fetal disorder. This method is already employed for Down's Syndrome screening (see Chapter 5), but new high throughput technology has accelerated the process of discovery for new biomarkers [Avent *et al.* 2008].

Although either of these technologies could potentially be applicable to NIPD, testing cffNA offers the most direct approach, and is therefore likely to be preferable wherever feasible. Ultimately, any merits these technologies might offer relative to DNA (and RNA) may be outweighed by practical considerations. Both cells and proteins require careful handling and may be extremely challenging both technically and logistically; in contrast, DNA is relatively simple to work with, in terms of extraction, storage and molecular detection, offering ease of transport and high reproducibility of results.

Fetal DNA has also been detected in maternal urine, which could potentially be tested for the purpose of prenatal diagnosis [Majer *et al.* 2007]. However, due to its low concentration and problematic detection, it is less appropriate for non-invasive prenatal diagnosis than cffNA in maternal blood and to date there has been significantly less research and clinical development in this area.

3.4 Ethical, legal and social issues

The concept of prenatal screening and diagnosis, and its association with termination, raises a number of ethical, legal and social issues (ELSI), which have been reviewed elsewhere [Scott 2003]. General issues associated with current antenatal care in the UK are not discussed here, as it is assumed that the current *status quo* is acceptable to the majority of society, and operates within a specific legal framework in which abortion is legal up to the 24th week of pregnancy, and after this date in some rare circumstances. Instead, the focus here is on issues specific to, or affected by, non-invasive prenatal diagnosis [Newson 2008]; application specific issues are dealt with in the relevant chapters. Notably, prenatal testing by means of cffNA raises novel issues by virtue of the fact that it crosses boundaries between professional prenatal testing, which has hitherto been governed by practitioners and institutions responsible for providing health care, and direct-to-consumer testing.

The ethical principles of autonomy, beneficence, non-maleficence and justice arguably provide a useful framework for analysis of the wider implications of NIPD. Within the context of routine antenatal care, it is self-evident that providing safer prenatal diagnosis would be beneficial in reducing harm, both to the mother and the fetus. However, several specific features of cffNA testing raise particular ethical and social issues, due to a likely increase in both testing and pressure to terminate, if NIPD were available:

- **Improved safety** - by removing any direct risk of harm to the fetus posed by invasive prenatal techniques, one of the current barriers to prenatal diagnosis is removed, which could result in an increase in the number of women opting for testing, and therefore facing a decision about termination. The technique is also safer for the mother, as unlike invasive techniques, it carries (almost) no risk of infection or physical trauma.
- **Earlier detection** - the ability to diagnose specific genetic disorders of the fetus within the first trimester, perhaps as early as 7 weeks gestation, means that parents have longer to make a decision about the future of the pregnancy. At this stage of pregnancy, earlier termination methods are available. However, this could result in an increase in the number of women opting for termination. In addition, since a proportion of abnormal fetuses will miscarry as the pregnancy progresses, earlier screening is likely to increase the number of abnormal fetuses both found and terminated.

- **Easier testing** - from the perspective of the patient, the practical simplicity of a single blood test for NIPD relative to invasive diagnostic testing or a multistage screening process may mean that some women may not make a properly informed choice, since its significance might not always be clearly understood or adequately explained prior to the test.

One of the major ethical challenges of implementing NIPD will be to support the autonomous choices of women. In the context of antenatal care, informed consent (whereby a patient is able to make independent decisions about their own health care) requires that the patient is fully informed about what tests are available, the consequences of having a test, what the results mean, and what options are available given each possible outcome of the test. In addition to appropriate provision of information, the patient must be allowed to make a decision in the absence of undue pressure or coercion.

Given the safety of NIPD, it is plausible to assume that many women might consent to prenatal testing; however, others might be less willing to consent, given the perception that testing might also lead to pressure to terminate. Additionally, the relative ease with which a blood sample can be taken for testing (along with numerous other standard tests performed at various points during the antenatal care pathway) could result in a lack of true consent, due to a failure to properly educate and inform the parents of the consequences of taking the test. Moreover, if cffNA-based tests represent a general shift from screening to diagnosis, it is unclear which of the two models traditionally associated with screening (informed choice) or treatment (informed consent) is more appropriate.

The requirement to provide ‘neutral’ information (for example, about genetic conditions that manifest with variable severity) on the basis of which a parent can make their own choice may be challenging and time consuming, in the context of both consenting to testing or choosing to terminate a pregnancy. Nevertheless promoting patient autonomy by safeguarding informed consent is a vital element of effective health care, and is currently recognised as a priority area by the National Institute for Health and Clinical Excellence (NICE) in its Antenatal Care Guidance [NICE 2008].

The future autonomy of the child must be considered when testing the fetus, particularly if testing for adult-onset conditions where current practice is to refuse testing until the child is competent to make their own autonomous decision about being tested. In addition, fetal nucleic acids extracted from the mother’s blood also have the potential to yield genetic information about the father, as is the case with traditional invasive prenatal diagnosis. Since currently only the pregnant woman is required to give consent for tests performed on her blood, ensuring respect for both paternal autonomy and the future autonomy of the child could therefore become increasingly challenging if NIPD becomes widely available without proper counselling and support.

A possible consequence of increased testing is more terminations, which could in turn result in increased social pressure to terminate, particularly if the diagnosed conditions were to become rarer in society resulting in a decline of support services (e.g. respite care homes for Down’s Syndrome families). In practice, it could become increasingly difficult for a patient who has received a positive test result *not* to ‘choose’ to abort. However, the use of NIPD as a form of ‘passive eugenics’ [Kitcher, 1997] is likely to become controversial only if it actually reflects a systematic undervaluing of the lives of the disabled. It is therefore important to ensure that policies in this area are genuinely motivated by concern for parental autonomy, rather than any sense of reducing financial and social ‘burden’, and that the experiences of disabled people and their families be fairly reflected when framing policy and educational materials.

It is conceivable that the technology will be applied to other conditions in future, and that this ‘specification creep’ may occur with or without medical justification or formal oversight. Notably, cffNA testing has already been used for prenatal sex determination in families at risk of haemophilia, many of whom would not seek invasive testing, as knowledge of fetal sex is useful for management of the birth. It has also been used as an alternative to preimplantation genetic diagnosis in assisted reproduction by couples who are at risk of specific inherited (sex-linked) diseases, ultimately resulting in the loss of more developmentally advanced fetuses. Whilst these specific applications may be medically justifiable, it is likely that an increasing number of applications could be offered simply because the technology makes them possible, rather than because testing is desirable or recommended.

Technical accuracy raises a number of issues regarding the beneficence and non-maleficence of NIPD. A false negative result could lead to failure to give treatment, or the birth of a diseased child, whilst a false positive result could lead to the termination of healthy pregnancies as well as unnecessary distress. Therefore, as with all new tests, the debate over the use of cffNA testing for NIPD should recognise that what seems to be a purely technical issue of maximising technical accuracy also has a complex ethical dimension.

Finally, the principle of justice requires that, if NIPD using cffNA is to be implemented in the NHS, it is crucial to ensure equity of access to the service, regardless of geographical location or ethnic variations. For technical reasons, it is possible that some diseases will never be capable of diagnosis through analysis of cffNA, and it is therefore important that the tests are presented from the outset in a way that reflects both their current and potential limitations in order to safeguard public trust in scientists and the medical profession.

In summary, NIPD using cffNA raises a number of ethical and social issues arising directly from some of the clinical advantageous properties of the technique (*i.e.* safer, earlier and easier). If this technology is to be effectively implemented throughout the NHS, these issues must be considered alongside the technical and clinical evaluation of the test and the practical implications for health service provision and workloads.

3.5 Non-clinical applications

Many of the possible cffNA tests have both clinical and non-clinical applications. For example, fetal sex determination can be useful clinically if the fetus is at risk of a sex-linked disease, but could also be used non-clinically for social sex selection. This is not only an issue within the NHS, where the line between medically and non-medically justifiable tests is rather indistinct, but also in the private sector. The increasing availability of non-invasive prenatal diagnostic tests direct-to-consumer (DTC) over the internet - currently primarily for the determination of fetal sex - suggests that the use of the technology for non-medical purposes could become widespread. This raises questions of whether policy should be developed to regulate access to these tests, and the extent to which this could be effective.

3.5.1 Sex selection

The biggest potential non-clinical application of cffNA is fetal sex determination in the absence of any specific medical need, both within the UK and further afield. The ethical and legal dimensions of sex determination and selection are complex. Within reproductive ethics, debates about sex selection have been characterised by three distinct positions:

- Extreme liberalism - sex selection is unproblematic, and reproductive autonomy is paramount;
- Extreme conservatism - sex selection is never ethically justifiable (with the possible exception of specific medical circumstances);

- Mixed position - sex selection is acceptable in some circumstances but not others, e.g. sex selection for the purpose of family balancing is acceptable unless it is driven by a sexist attitude that women are inferior to men.

Whilst the moderate view is appealing, because it tries to steer a middle path between the two extremes, theoretical and practical difficulties remain. Moreover in a pluralistic society, it may be challenging to achieve consensus on what counts as a legitimate reason for sex-selective termination. Even if consensus can be achieved, the interface between prenatal sex determination and the law regulating sex selection and abortion is complex. Additional regulation applies to the selection of embryos for assisted reproduction using pre-implantation genetic diagnosis, for which a licence from the Human Fertilisation and Embryology Authority (HFEA) is required. The selection of embryos on the basis of sex is not currently permitted, except where the justification is to reduce the risk of gender-related serious disability, or illness or condition. This regulatory position is endorsed by the Human Fertilisation and Embryology Act, in which the powers to authorise sex selection are limited to grounds relating to the health of any resulting child [Human Fertilisation and Embryology Act 2008].

However, having identified the sex of her fetus, there is no prohibition upon a woman seeking the termination of pregnancy (before 24 weeks) under the Abortion Act 1967 if two medical practitioners agree that *'continuation of the pregnancy would involve risks... of injury to the physical or mental health of the pregnant woman'*. The major ethical concern in this area is therefore that prenatal fetal sex determination, in combination with termination of pregnancy, could result in sex selection for non-medical or 'trivial' reasons, which could have major implications for society.

3.5.2 Testing for minor fetal abnormality

In the longer term, NIPD based on cffNA may become available for numerous applications that have no clear clinical benefit, such as susceptibility to complex diseases or other genetic traits. The effect of a positive result may be difficult to interpret, given that future development of the disease may be contingent upon environmental factors or inheriting other susceptibility genes (for which testing may not be available). This begs the question of how women should interpret the results of these tests. If they wish to terminate the pregnancy (on grounds of fetal abnormality), they may be unable to do so unless they are able to persuade two medical practitioners that *'there is a substantial risk that if a child were born it would suffer from such physical or mental abnormalities as to be seriously handicapped'* (Abortion Act 1967, 1(1)(d)), although termination of the pregnancy would still be an option before 24 weeks under the 'social ground' of the Act [Scott 2005]. The law encourages a lack of transparency about the justifications for termination of pregnancy, and capturing the number of pregnancies that are terminated, and the grounds upon which they are terminated may be important in the future, given the likely increase in the scope and power of prenatal genetic testing.

3.5.3 Prenatal Paternity Testing

In principle, the technology could be used for paternity testing, by comparing the non-maternal DNA from a maternal blood sample with specific DNA sequences sampled from the putative father [see Wagner *et al.* 2008 for proof of principle]. Within the UK, paternal consent for testing is required under the Human Tissue Act 2004, reinforced by a voluntary draft code of practice. This code recommends that women seeking prenatal paternity testing should be advised to contact their GP or midwife before submitting the test, so that they can obtain appropriate independent counselling. However, the availability of commercial 'forensic' paternity testing services via the internet, which utilise samples of DNA from common materials such as cigarette butts and hair, make this impossible to enforce in practice.

3.6 Implications for developing countries

Outside of the UK, there are numerous wide-ranging implications of NIPD using cffNA testing. The most obvious is the non-clinical use of cffNA for fetal sex determination and, coupled with termination, sex selection for social reasons. The extreme population skewing that has occurred in China and India as a result of unlawful (pre- and postnatal) sex selection practices, favouring male children, has raised concerns that prenatal fetal sex determination using NIPD could be used for sex discrimination and aggravate this problem. Although sex determination using ultrasound is illegal in certain places as a result of this population skewing, the emergence of cffNA tests available directly to the consumer, without the involvement of a clinician, could have a significant effect. Moreover, whilst such an extreme situation is unlikely to occur in other countries, concerns about the use of the technology for non-medical reasons might distort equity of access to NIPD for different ethnic groups throughout the world, which in turn might unfairly propagate ethnic or racial stereotypes in society.

There are also significant implications for clinical applications of cffNA testing in the developing world. Congenital and genetic disorders are recognised as a growing health problem in many developing countries, as the health status of populations increases and infant mortality falls [Alwan & Modell 2003]. Haemoglobin disorders and Down's Syndrome are among the common birth defects affecting developing countries, and can have devastating consequences in the absence of the relevant support services. It is likely that as many developing countries begin to address the prevention of birth defects, cffNA testing may prove to be an appealing option due to their cost effectiveness, relative ease of use and convenience in comparison with conventional methods of promoting parental choice.

In comparison with conventional prenatal testing processes, such as amniocentesis, CVS and ultrasound, cffNA tests are significantly less technologically intensive at the point of care, requiring only a routine blood sample. This has a number of advantages, including negating the need for specialist equipment and technicians for sample collection and cytogenetic analysis. The process can be carried out by local health professionals and may be preferred due to the reduced risk of passing on infections as well as savings in time and service provision. In addition, DNA is more stable than cellular material, so in theory samples can easily be sent to designated specialist centres for analysis, making testing more widely accessible across different countries. However, the need for rapid sample processing (e.g. centrifugation and freezing) prior to transportation could cause problems in countries where there is little funding or service provision.

It is likely that private health care providers in developing countries will incorporate cffNA testing into clinical practice in much the same way as in the developed world, although the manner and extent of introduction may be dictated by the infrastructure they already have in place. The use of these tests by national health service providers to the general population is more difficult to predict; although this technology could have a large impact on clinical genetic services in developing countries, its uptake will be influenced by the infrastructure and policy required to integrate genetic technologies into the existing health service frameworks.

The provision of both genetic and prenatal screening/diagnostic services in developing countries is currently very variable, due to the heterogeneity in service provision both within and between countries. Systematic screening programmes are often lacking and provisions for diagnostic testing are sparse. In addition, regulation of abortion and the role of patient autonomy within medical care vary enormously between countries, and there is significant variation in the realisation of informed choice [Van den Heuvel *et al.* 2008]. Moreover, the availability of NIPD in countries where access to safe termination of pregnancy is absent could cause significant social and medical problems [Ballantyne *et al.* 2008]. For example, there is wide variability in those countries offering abortion on socio-medical, economic or social grounds; this is permitted in approximately 78% countries in the developed world and 19% countries in the developing world [United Nations 2007].

3.7 Intellectual property issues

There are multiple patents in this area covering both innovative new products (e.g. isolated fetal DNA extracted from the maternal blood), new methods (e.g. technologies for diagnosis) and new uses (e.g. various specific applications of non-invasive prenatal diagnosis). The first key piece of intellectual property (IP) in this area, European patent EP 0994963B1, is owned by the University of Oxford's ISIS Innovations Ltd (with Dennis Lo as one of the inventors). It was filed in 1997 and subsequently granted in Europe (including the UK) in 2003; patents were also granted in the US and elsewhere.

The European patent includes broad claims to *'a detection method performed on a maternal serum or plasma sample from a pregnant female, which method comprises detecting the presence of a nucleic acid of foetal origin in the sample.'* The claims also cover *'a method of performing a prenatal diagnosis which comprises: (i) separating a maternal blood sample into a cellular and non-cellular fraction; (ii) detecting the presence of a nucleic acid of foetal origin... and (iii) providing a diagnosis based on the presence and/or quantity and/or sequence of the foetal nucleic acid.'* Clinical applications specifically listed include: fetal sex (through detecting a Y chromosome sequence); RhD status (in a RhD negative mother); chromosomal aneuploidy (specifically Down's Syndrome); detection of paternally inherited non-Y chromosome nucleic acids; and diagnosis of pre-eclampsia.

In 2005, ISIS granted an exclusive licence to the US-based company Sequenom® Inc., giving it extensive rights to control the technology claimed in its European and US patents. The licences cover all uses of the technology, with the exception of the use of QF-PCR for RhD genotyping in Europe, which had previously been exclusively licensed to L'Institut de Biotechnologies Jacques Boy in France. Financial terms of the licence include up-front fees, milestone payments and royalties on product sales. Sequenom has also licensed exclusive international rights relating to cffRNA, and also owns IP relating to various relevant technology platforms, including mass spectroscopy and digital PCR, demonstrating considerable geographical scope and technical diversity.

Numerous other patents referring to specific applications and relevant developments in NIPD, such as cffRNA, are also owned by or exclusively licensed to Sequenom. Recently, the US biotechnology company Fluidigm® announced that it had secured co-exclusive licenses (with an unidentified prenatal diagnostics partner company) to inventions at Stanford University that detect fetal genetic characteristics in maternal plasma (press release, 1st December 2008), including the use of a combination of digital PCR and high-throughput sequencing. The claims of the patent WO 2007092473A2 specifically include the non-invasive detection of fetal Down's Syndrome from maternal plasma, as well as numerous genes relating to specific single gene disorders. It is currently unclear whether or what actions Sequenom can and will take in response to this patent.

From the perspective of the NHS, it would be prudent to make the assumption that the entire area of NIPD using cffNA testing is (or will be) covered by extensive and commercially valuable intellectual property rights. Hence, anyone making, using, keeping, selling or importing the technology covered in the patent claims in any of the designated countries without permission from the patent owner would be infringing the patent (Section 60 of the UK Patent Act 1977). There are several exceptions to this rule. Of most relevance are the rules found in most European countries that no liability arises if the infringing activity is *'done privately for purposes which are not commercial'* (e.g. individuals) or for *'experimental purposes relating to the subject matter of the invention'*, i.e. bonafide research. The latter exception is extremely relevant to the development and clinical evaluation of cffNA testing within a research setting, during which time the NHS would not be infringing the patent so long as the purpose and scope of the research is adequately evidenced and justified.

If the technology were to become offered as a service (in a commercial or business manner, including public services), this exception would no longer apply and, in the absence of a permission or licence, the service provider would be infringing the patent. In such a case, there are number of different options available to the patent holder, including ignoring the use, offering a commercial service to test users (such as sale and supply of hardware or test kits), negotiating a licence, and suing for infringement. The relative likelihood of each of these options will most likely vary with the clinical application and country. If the patent holder refuses to negotiate a *reasonable* licence, a user may exert a right to apply for a compulsory licence. The compulsory licensing provisions in the UK effectively state that, after three years from the date of the grant of the patent, the patent owner cannot refuse to grant a licence on reasonable terms to use the technology. For these reasons, in addition to the associated costs and possibility of successful legal challenges to the claims, in practice litigation is very rare. Anti-competition law also states that the patent owner cannot ‘*abuse*’ a dominant market position. Furthermore, if reasonable attempts have been made to inform the patent owner of test usage and are met with silence, it is unlikely that the patent owner could later claim not to have noticed the infringement and claim compensation retrospectively. Therefore, although the NHS will probably have to pay for the right to use cffDNA testing, it is highly unlikely that the IP situation will ultimately be prohibitive to offering NIPD in the UK.

3.8 Commercial situation

There are a number of companies currently active in this area. The largest and most dominant company is Sequenom Inc., a California-based genomics and diagnostics company that holds an exclusive licence to most of the existing IP related to the application of cffDNA for NIPD. The tests are primarily based on the company’s proprietary mass spectrometry technology. Although the patents cover NIPD for sex determination and certain single gene disorders, Sequenom’s primary focus is on the potentially significantly larger Down’s Syndrome testing market.

Sequenom is not a DTC company, and therefore is likely to make or license the tests to laboratories. In December 2007, it introduced a commercially available *RHD* and *SRY* genotyping test for RhD incompatibility in the US through a commercial partner, Lenetix® Medical Screening Laboratory, which sells to health care providers. The Down’s Syndrome NIPD test is still in development, but Sequenom is aiming for a US launch date in June 2009, pending the results of further clinical trials (press release, 23 September 2008). The test will be offered as a screening test prior to invasive diagnostic testing, and is currently intended to be available through physician referral only.

Several companies currently advertise NIPD direct-to-consumer over the internet, the majority based on a maternal blood prick sample:

- L’Institut de Biotechnologies Jacques Boy in France sells a *Free DNA Fetal Kit® RhD*; the test costs €1,653 for 90 reactions.
- Acu-Gen Biotech Inc. offers *Baby Gender Mentor™* (from 5 weeks) for fetal sex based on cffDNA; the test costs \$275.
- Consumer Genetics Inc. offers the *Pink and Blue®* Early DNA Gender Test (from 7 weeks) based on cffDNA; the tests costs \$244.
- DNA Plus offers both a fetal sex (from 10 weeks) and paternity test (from 13 weeks) based on circulating fetal cells in the mother’s blood; the tests cost \$390 and \$990 respectively.
- Urobiologics offers the *Urogender* test for fetal sex (from 5 weeks) using a urine sample; the test costs \$275.

Regulation of the burgeoning DTC genetic test sector has been widely debated, and although many are critical of the test quality, reliability and the lack of support available to applicants, it is unclear what can be done to protect the consumer given that there is significant disagreement about the degree of statutory regulation required. However, it is unlikely to be possible (or indeed desirable) to simply ban any DTC tests that are accessible via the internet. In its recent report *More Genes Direct*, the UK Human Genetics Commission instead recommended that a voluntary code of practice relating to genetic testing services be developed to ensure appropriate standards are being met [Human Genetics Commission 2007]. This has recently been followed up by a proposal for the development of a 'Common Framework of Principles for Direct Genetic Testing' (HGC08/P16), which would lay down some high level overarching principles that could be applicable across jurisdictions. However, the mechanism for regulation and achieving compliance with any voluntary code is unclear.

NIPD tests raise a somewhat different set of difficulties from standard DTC genetic tests (which are largely predictive susceptibility tests) because of their potentially diagnostic nature and the fact that a non-consenting party that is not afforded personhood under current UK law (*i.e.* the fetus) is actually the subject of the test. The European *In Vitro* Diagnostics Directive (IVDD), which is upheld by the Medicines and Healthcare Products Regulatory Agency (MHRA) in the UK, requires that devices used for self-testing must be safe and supported by clear instructions. These should provide advice on the action to be taken in response to a positive, negative and indeterminate result, rates for false positive and negative results, and a statement directing the user not to take a decision of medical relevance without first consulting their medical practitioner [IVDD 1998, section 8.7(t)]. Prenatal kits are likely to be classified as 'low risk' regardless of the potential for a woman to use information from the test to decide whether or not to continue the pregnancy. Although access to terminations in the UK is through physicians, in practice a woman could seek a termination for social reasons (prior to 24 weeks) based on the results of a DTC test.

3.9 Economic considerations

A full economic evaluation of NIPD based on cffNA is outside the scope of this report. However, cffNA testing itself is relatively inexpensive, and the cost is continuing to decrease as new high throughput genetic technologies are developed.

A general impression of the economic impact of cffNA testing can be made by considering the proportion of tests that would change the clinical management of pregnancy, and the effect(s) of this change within each of the main applications. One of the expected outcomes of prenatal cffNA testing is a decrease in the number of invasive diagnostic tests. The cost of amniocentesis or CVS is around £500, compared with a negligible cost of taking a blood sample. Sample processing costs relating to culturing the fetal cells for karyotyping are an additional £150, whilst DNA extraction and analysis using molecular genetics techniques costs around £250; this last figure is likely to be comparable to the costs of cffNA testing [Norbury & Norbury, 2008]. Therefore, testing using cffNA will be significantly cheaper than invasive diagnostic testing - perhaps less than half the cost - as the molecular laboratory techniques required are comparable (assuming molecular techniques are used for analysis of the fetal material), but the invasive procedure itself is avoided.

Ultimately, a full economic assessment will be required for each application, the outcome of which will be highly dependent upon the purpose of testing. The cost effectiveness of using cffNA testing will depend not only upon the cost and accuracy of laboratory analysis, but also on a number of wider economic factors, such as cost per procedure-related miscarriage avoided, quality adjusted life years comparisons for each testing option and disease outcome, changes to the clinical care pathway, and hospital resources and staffing.

4 High genetic risk families

Pregnancies in which there is a known family history of an inherited genetic disorder are classified as high risk, and referred as early as possible to genetic specialists. These families usually seek medical advice early in pregnancy, or even before conception, giving them the possibility of an early and unhurried diagnosis with subsequent time for decision making. According to the Clinical Molecular Genetics Society Audit 2005-06, around 1,500 (invasive) prenatal diagnoses are performed in the UK *per annum* by the network of regional molecular genetic laboratories (excluding those resulting from aneuploidy screening).

Currently, cffDNA testing is applicable to two distinct classes of familial genetic disorders, and could eliminate the need for invasive testing in around half of cases:

- (1) sex-linked disorders (and certain endocrine disorders relating to the development of ambiguous genitalia);
- (2) single gene disorders (primarily those in which the maternal and paternal alleles differ).

4.1 Sex determination

4.1.1 Current Practice and Epidemiology

Most sex-linked diseases are recessive X-linked diseases caused by a particular mutation on the X chromosome. The disease therefore normally only manifests itself in males (who carry a single X chromosome), whilst in females the diseased allele is compensated for by the normal allele present on the other X chromosome. Male offspring of women who are carriers of an X-linked disorder have a 50% chance of inheriting the condition. Whilst each disease is individually relatively rare, it has been estimated that combined they occur in around 0.5 per 1,000 live births [Baird *et al.* 1998]. Relatively common X-linked recessive diseases include haemophilia (a blood clotting disorder) and Duchenne muscular dystrophy (a progressive muscle wasting disease), although there are numerous others often resulting in severe phenotypes.

Women with a family history of a sex-linked disease may either self-refer prior or during pregnancy, or may be urgently referred by their GP or obstetrician. Classically, fetal diagnosis is done using invasive genetic testing, and although ultrasound can be used as a guide for fetal sex, it is not accurate enough early in the first trimester to be used diagnostically in high risk cases (~70% accurate at 11 weeks, and 99+% accurate at 12+ weeks) [Efrat *et al.* 1999]. Accurate determination of sex non-invasively could therefore reduce the number of invasive diagnoses required for each specific disease by half (female fetuses would not require further testing, but male fetuses would still require invasive testing to determine whether they had actually inherited the disease carrying allele from the mother). There is also clinical justification for early sex determination in certain endocrine disorders, such as congenital adrenal hyperplasia (which has a prevalence of around 1 in 15,000), in order to target antenatal hormonal treatment and prevent masculinisation of a female fetus.

Although proposals for non-invasive prenatal determination of fetal sex using cffDNA currently only include cases where an adverse clinical indication is suspected, the inclusion of cases where invasive testing is not currently offered routinely, suggests that fetal sex determination could potentially be used more widely in the future. This emphasises the need for careful justification of the introduction of the technology into new clinical situations.

4.1.2 Status of cffNA testing

Due to the relative ease with which the Y chromosome of a male fetus can be distinguished from maternal DNA, the most common clinical application of NIPD using cffDNA to date is fetal sex determination. Proposals for cffDNA testing to determine fetal sex within the NHS are limited to those cases where an adverse clinical indication is suspected, specifically where the fetus is at risk of inheriting:

- an X-linked disorder, including cases where invasive testing is not usually offered (such as haemophilia), in order to reduce the number of invasive tests required;
- certain endocrine disorders (such as congenital adrenal hyperplasia), where development of external genitalia is ambiguous and masculinisation of a female fetus is preventable with antenatal treatment; early fetal sex determination could have significant utility in avoiding or reducing unnecessary exposure to dexamethasone treatment in male fetuses.

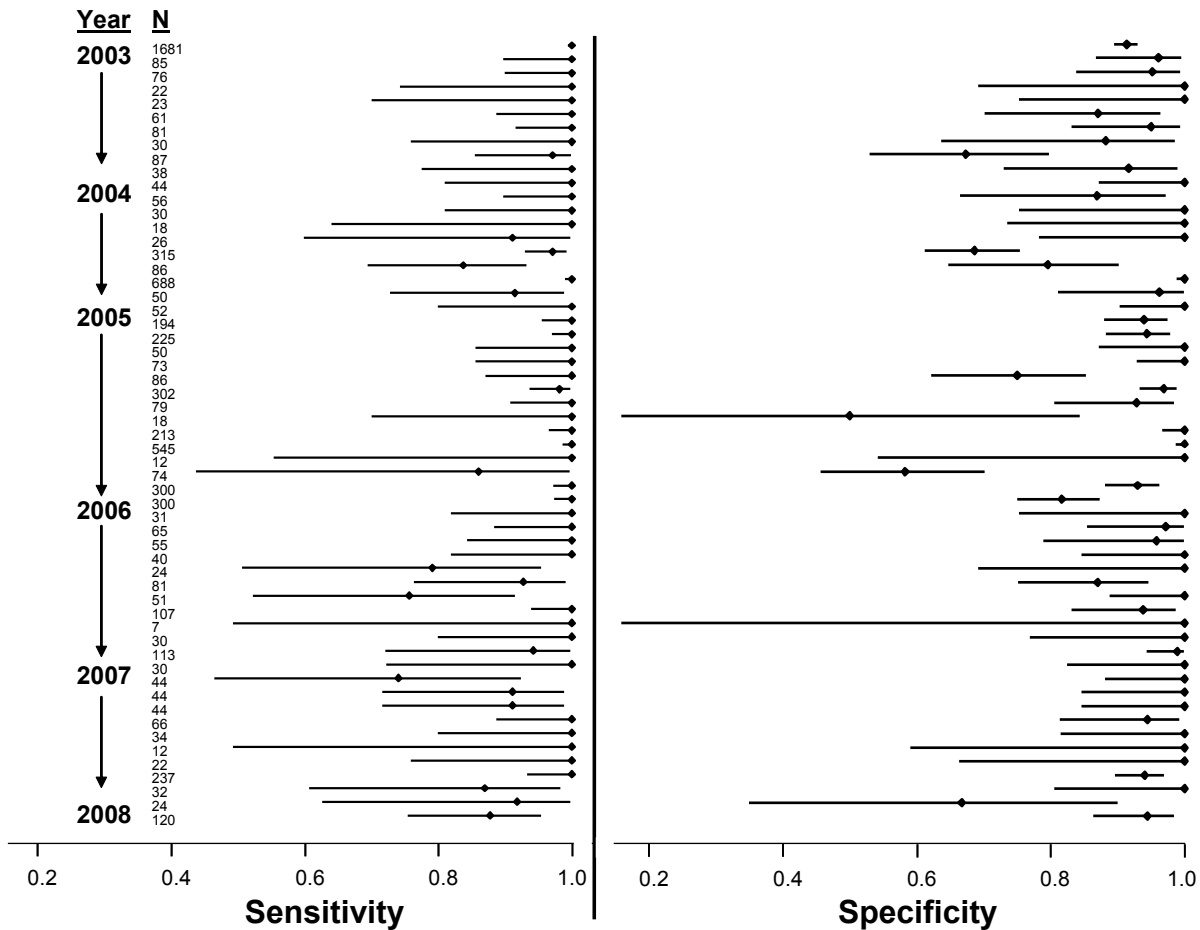
The majority of studies using cffDNA for sex determination use real-time QF-PCR to detect genes on the Y chromosome of male fetuses; most detect the sex-determining region Y (*SRY*), although a number of other Y chromosome specific sequences present in multiple copies per male genome have also been investigated (most commonly *DYS14*). Female fetuses are not detected directly, but inferred by a negative result for the Y chromosome, which could also be caused by undetectable levels of cffDNA or laboratory failure. Therefore, the method is inherently prone to false negative test results, *i.e.* male fetuses wrongly said to be female. A number of investigators have tried to address this issue by simultaneously testing for universal fetal markers as a positive control for the presence of cffDNA. In contrast, false positive tests, *i.e.* female fetuses wrongly said to be male, can be significantly minimised by controlling for contamination with non-fetal male DNA.

There are multiple studies which describe the accuracy of using cffDNA to determine fetal sex prenatally [Finning & Chitty 2008] and a systematic review is currently ongoing². A preliminary meta-analysis, limited to the 57 most recent publications (between Jan 2003 and Feb 2008) including over 7,000 test results, produced an overall clinical sensitivity of detecting a female fetus of **99.9%** (95% confidence interval 99.7-100.0%) and a clinical specificity of **94.9%** (95% confidence interval 93.5-96.4%) (Figure 4-1); these estimates do not change significantly when the analysis is limited to data from the first trimester only. The data also suggest that the accuracy of the technique may be somewhat lower prior to 7 weeks gestation.

Prenatal sex determination using cffDNA is currently available in a number of laboratories in the UK, including the International Blood Group Reference Laboratory in Bristol and the North East Thames Regional Genetics Laboratory at Great Ormond Street Hospital in London. A recent audit of tests performed from March 2006 to April 2007 revealed that of the 160 women tested, the accuracy was 97.6% and the number of invasive tests was almost halved [Chitty 2007]. Of these tests, 77% were requested as a result of suspected X-linked diseases, including 22% relating to haemophilia for which invasive prenatal diagnosis is infrequently requested.

² Collaboration between the University of Cambridge MRC Biostatistics Unit and the PHG Foundation.

Figure 4-1 Preliminary results from an ongoing systematic review and meta-analysis of prenatal fetal sex determination using cffDNA.
(Limited to publications between Jan 2003 and Feb 2008)



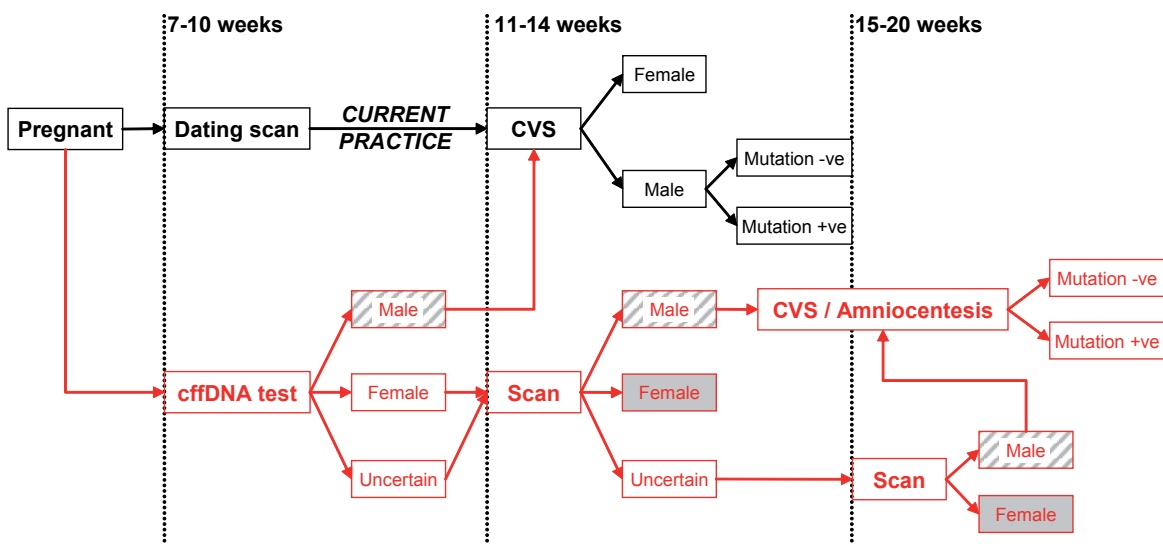
Key: diamond = point estimate; line = 95% confidence interval; N = total number of tests; sensitivity = proportion of female fetuses correctly identified; specificity = proportion of male fetuses correctly identified.

It has recently been suggested that some females could be wrongly diagnosed as male due to the presence of a vanishing (male) twin, and it has been estimated that this could produce a false result in around 0.3-0.7% of pregnancies³ [Gail Norbury, personal communication]. In order to reduce this problem, it has been suggested that all cffDNA testing for fetal sex should be accompanied by an ultrasound scan, which could be done early in pregnancy, as loss of the twin usually occurs in the first seven weeks [Landy & Keith 1998]; however, the identification of a vanishing twin on ultrasound would necessarily make the results of a cffDNA test ambiguous, even though the results would only be discordant in 25% of cases.

³ Limits of estimation based on two calculation methods: (1) an estimated twinning rate of 5.4%, of which approximately half will be dizygotic and 40% can be expected to vanish; (2) a live-born twinning rate of 1.3%, of which two-thirds are dizygotic and assuming that only a third of twins survive until birth. Note that only one in four possible twinning scenarios will cause a false result, namely where there is a female fetus and a male vanishing twin.

The fact that the female fetuses are not directly detected, but inferred from a negative Y chromosome assay, may cause concerns amongst clinical geneticists who wish to confirm that the fetus is female with complete certainty, in order to rule out the possibility of X-linked diseases and therefore the need for further invasive diagnostic testing. The main implications for this test in a clinical pathway are that invasive diagnostic testing can be avoided only in the case of a female fetus. Where a female fetus is incorrectly classified as male (*i.e.* Y-chromosome DNA is wrongly detected), there would be no change to the current clinical pathway; however, invasive testing would be delayed in the cases where a male fetus is incorrectly classified as being female (*i.e.* due to failure to detect Y-chromosome DNA), resulting in a reduced quality of care (see Figure 4-2). Therefore, the specificity of detecting a female fetus is of primary importance for assessing the impact of the test on patient care.

Figure 4-2 Suggested clinical pathway for the use of cffDNA testing in pregnancies at risk of an X-linked disease.



Key:

Current practice in black; addition of fetal genotyping in red.
 Pregnancies where invasive testing is avoided indicated by shaded boxes.
 Pregnancies where invasive testing is delayed indicated by striped boxes.

4.2 Single gene disorders

4.2.1 Current Practice and Epidemiology

Thousands of rare human diseases are caused by mutations in just a single gene and it has been estimated that their combined occurrence is around 3.6 per 1,000 live births [Baird *et al.* 1998]. Single gene disorders on the autosomal chromosomes can be divided into two categories based on their pattern of inheritance:

- *dominant* diseases are caused by a single copy of the defective gene, which is inherited from only one parent, *e.g.* Huntington's disease;
- *recessive* diseases are caused by two copies of the defective gene, which must be inherited from both parents, who are termed carriers, *e.g.* sickle cell anaemia and cystic fibrosis.

Women with a family history of a particular monogenic disease may either self-refer to a clinical geneticist prior to or during pregnancy, or may be urgently referred by their GP, midwife or obstetrician. Currently, fetal diagnosis is done using invasive genetic testing.

Pregnancies at high risk of certain common autosomal single gene disorders may also be identified through National Screening Programmes, such as the antenatal NHS sickle cell and thalassaemia carrier screening programme. These diseases, which are most prevalent in African and Asian populations, account for around a third of the prenatal diagnoses performed in the UK *per annum* (excluding tests for the common aneuploidies) according to the Clinical Molecular Genetics Society Audit 2005-06.

4.2.2 Status of cffDNA testing

Detection of fetal point mutations from a sample of maternal blood is extremely technically challenging, due to the predominance of very similar maternal DNA sequences. Enrichment of cffDNA (through size fractionation) is generally required to increase the sensitivity of detection, which makes it challenging to calculate the dosage (*i.e.* number) of any homozygous mutations. Moreover, detection of large-scale mutations (*e.g.* disorders caused by expansion, insertion or duplication of sequences) is restricted to sequences less than ~300 base-pairs in length, due to the fragmented nature of cffDNA. Given these technical limitations, there have been a limited number of studies published using cffDNA testing for NIPD of single gene disorders, and those that have been published generally include only a small number of cases [see Norbury & Norbury 2008 for review].

The most obvious application is the detection of paternally inherited dominant diseases, to which cffDNA analysis has already been applied for prenatal diagnosis of Huntington's disease, achondroplasia, torsion dystonia, Aperts syndrome and myotonic dystrophy. Applying a similar methodology to autosomal recessive diseases, NIPD based on cffDNA can be used to determine the carrier status of the fetus in compound heterozygotes, *i.e.* through detection of a paternally inherited disease allele in cases where multiple disease alleles are known and the maternal and paternal inherited allele differ. In such cases, cffDNA testing could be used to identify fetuses that have not inherited the paternal disease allele, and hence are not at risk of having the disease, thus reducing the number of invasive diagnoses required by approximately 50%. However, compound heterozygosity is known to account for only a small percentage of recessive diseases, and to date, fetal carrier status has been determined using cffDNA testing for cystic fibrosis, β -thalassaemia, Hb Lepore and congenital adrenal hyperplasia (for which fetal sexing could be used instead).

This methodology has specific ramifications for the NHS Sickle Cell & Thalassaemia Screening Programme. Around 25% of prenatally diagnosed β -thalassaemia is caused by compound heterozygosity [John Old, personal communication], so cffDNA testing could be used to reduce the number of invasive diagnostic tests resulting from screening. However, because all cases of sickle cell anaemia (and the majority of cases of thalassaemia) are caused by homozygous mutations, it is unlikely that this type of cffDNA testing will have a major impact on population screening for these diseases.

In the longer term, it may be technically feasible to diagnose significantly more single gene disorders, including those caused by homozygous autosomal recessive mutations, by determining the relative dosage of the disease causing allele versus the normal allele. This requires significant enrichment of fetal DNA prior to detection, coupled with a highly sensitive detection platform capable of assessing gene dosage with high discrimination power. A preliminary study showing proof of principle for this method has been published [Lun *et al.* 2008b], which uses modified digital PCR to measure the 'relative mutation dosage' of the gene of interest, *i.e.* the ratio of the diseased to the normal allele. This method exploits the fact that cffDNA fragments are shorter than maternal DNA to selectively amplify fetal DNA specifically in the region of interest. However, this technique still requires extensive refinement, development and validation, and its widespread clinical application is unlikely to occur within the timescale considered by this report.

It should also be noted that disease susceptibility variants, such as *BRCA1/2* mutations in breast cancer, could conceivably be added to the list of potential single gene mutations that could be prenatally diagnosed using cffDNA technology. However, to date, this has not been reported.

4.3 Ethical, legal and social issues

Families at high risk of an inherited genetic disease are likely to access cffNA testing via the clinical genetics service, which already has well-established systems for ensuring informed consent. Since cffNA testing enables the earlier diagnosis (or elimination of the need for diagnosis) of single gene conditions, the most significant ethical issue in this area is the combination of NIPD with selective termination, on the grounds of fetal abnormality, to promote a form of 'passive eugenics'. The increasing ease and availability of prenatal diagnosis may be taken to implicitly suggest under-valuation of the lives of those living with disabilities, and hence be regarded as a form of discrimination against those living with a disability. Even assuming that the fall in disability rate is not an intended outcome of state policy, members of the disability lobby claim that policies which would foreseeably reduce the rate of disability are inherently wrong, as people living with disability can lead perfectly decent lives, and the use of such technologies will result in increased stigmatisation against the disabled. However, in this respect NIPD is not qualitatively different from current technologies, as the outcome of both is to offer the at-risk couple an informed reproductive choice without the additional risk of an invasive procedure.

If NIPD is extended to a range of conditions for which invasive testing is not routinely offered, this would require ethical and clinical justification. The possibility of specification creep (where cffNA tests might be used without adequate clinical justification) could also apply to prenatal testing for diseases that are likely to arise in adult life, or where testing is done to predict future susceptibility to disease. The addition of tests for increased risk of developing adult-onset diseases is significantly more ethically problematic than testing for inherited or congenital disorders, particularly as in the former case, the person may never actually develop the disease and might prefer not to know about it. There has already been extensive discussion of this subject in the area of pre-implantation genetic diagnosis and assisted reproduction [Robertson 2003].

The specific ethical issues raised by non-clinical uses of cffDNA testing for fetal sex determination, such as family balancing, are dealt with in *Section 3.5*.

4.4 Perspectives

4.4.1 Patients

Many women with a family history of a genetic disorder go directly to a clinical geneticist, and are often well informed about the choices available to them and the consequences of these choices. Although the psychological and emotional impact of earlier diagnosis are unclear, there is little doubt that NIPD would be popular amongst these patients as an alternative to invasive diagnostic testing, as it poses no direct risk to the fetus or mother and can be performed early in the pregnancy. It is worth noting that the majority of calls received by a helpline run by the UK charity Antenatal Results and Choices (ARC) relate to concerns over invasive testing. Moreover, there is likely to be a demand for private services if particular cffDNA tests are not available on the NHS, though it is unclear to what extent commercial services would be limited to clinical applications. It is important, particularly for managing the expectations of patients, to emphasize that not all prenatal diagnosis will become non-invasive in the near future.

4.4.2 Physicians

High risk families are generally dealt with by clinical geneticists and obstetricians, so it is these two professional groups who would primarily be involved in offering and explaining the test to patients. Currently, the awareness of tests based on cffDNA amongst obstetricians may be relatively low, and there is some scepticism about the accuracy of the technique amongst those who are aware of it. In cases such as sex determination, the diagnosis can be confirmed by ultrasound; however, such 'back-up' processes are not available for single gene disorders. Very little change would be required in the clinical referral pathway, other than requesting a NIPD test prior to (or instead of) an invasive test.

The use of cffDNA testing for fetal sex determination for non-medical purposes is likely to generate mixed reactions amongst the medical community, who will nonetheless probably have to deal with patients who have obtained the test privately. However, the Royal College of Obstetricians and Gynaecologists released a statement in 2007 that it "*strongly believes that sex selection for non medical purposes is inappropriate... focus should remain firmly on the health and care of the mother and developing baby, rather than gender*".

4.4.3 Laboratories

Currently, results from invasive diagnostic testing from pregnancies at high risk of a specific genetic disorder are usually processed either by molecular genetics or cytogenetics laboratories. Since invasive testing cannot be entirely avoided, it will be important to maintain this skill base.

Due to the relatively low throughput, the addition of a cffNA test should not require a major change in workflow, expertise or equipment for the molecular genetics laboratories. Currently, prenatal testing for single gene disorders is undertaken by many different laboratories, all of whom do specialist testing. It is unclear whether this situation would continue for cffNA tests, with different specialist laboratories offering tests nationally for specific diseases, or whether local laboratories would offer all the available tests.

4.4.4 Commissioners

In families at high genetic risk, the potential change in the management of the pregnancy would be a reduction in the number of invasive diagnoses required. Using currently available methodology, it is likely that cffDNA testing could reduce the number of diagnostic tests required by around 50%. Therefore, given that cffDNA testing is currently less than half the cost of invasive testing, we would expect there to be a slight reduction in the overall cost of antenatal care for pregnancies at high risk of inherited genetic disorders as a result of cffDNA testing.

There are a number of organisations currently involved in the evaluation and commissioning of genetic tests specifically for single gene disorders, all of whom require a substantial evidence base in order to recommend that a test be made available nationally.

The UK Genetic Testing Network (UKGTN) advises the NHS on genetic testing for inherited disorders. It is a voluntary network of genetic testing laboratories that aims to ensure the provision of high quality equitable genetic testing services for all NHS patients across the whole of the UK. This involves evaluating new tests and making recommendations to commissioners on new NHS services. It has developed a pragmatic method for evaluating new tests, whereby laboratories must complete a Gene Dossier for each new test in order for it to be listed in the UKGTN database and be available to the network. The criteria used in test evaluation are based upon the ACCE Framework⁴ developed by the US Centres for Disease Control [Haddow & Palomaki 2004]. Specifically, the areas to be covered in the gene dossier include:

- (1) the seriousness of the condition;
- (2) the prevalence of the condition;
- (3) the purpose of the test, *e.g.* diagnosis, treatment, prognosis, management, presymptomatic testing, risk assessment, *etc.*;
- (4) the laboratory details and complexity of the test;
- (5) the context in which the test is to be used, including different populations;
- (6) the clinical characteristics of the test, *i.e.* its clinical sensitivity, specificity and predictive values;
- (7) the clinical utility of the test, *i.e.* the benefit to the patient given the availability of alternative diagnostic procedures;
- (8) ethical, legal and social considerations;
- (9) the cost of the test.

A separate gene dossier must be completed for every new test, whether the novel component is the technology, the disease or the intended purpose. For example, a generic test for fetal sex would not be acceptable without a specific purpose, *e.g.* assessing risk of a specific (named) sex-linked disease, and consideration of the clinical care pathway and context in which it would be used. There might need to be some centralised process by which labs agree who would take the lead on obtaining a gene dossier for each application, and ultimately the capacity of the UKGTN to review applications might be a limiting factor

The Genetics Commissioning Advisory Group (GenCAG) is an advisory group, set up by the UK Department of Health, which is involved in commissioning genetic tests where nucleic acid is the analyte. It takes a strategic national overview of genetics in health care delivery and oversees the UKGTN steering group, holding the group and the co-ordinating team to account for delivery, setting strategic direction for their work and making decisions where necessary.

⁴ A model developed for evaluation of genetic tests, including the analysical validity, clinical validity, clinical utility, and the ethical, social and legal implications of the test.

5 Routine antenatal screening

All pregnant women are offered routine antenatal screening for numerous diseases, including various infectious diseases and fetal anomalies (see National Screening Committee website for full details, www.nsc.nhs.uk). There are a number of criteria for appraising the viability, effectiveness and appropriateness of a screening programme, including the disease severity and epidemiology, the parameters of the test (such as safety, accuracy, level of evidence, acceptability, *etc.*), the effectiveness of any interventions or treatments, and the overall benefit, acceptability and cost-effectiveness of the screening programme.

The main application where cffNA technology could potentially be introduced within the current routine antenatal screening programme is testing for Down's Syndrome and other chromosomal aneuploidies.

5.1 Aneuploidy

5.1.1 Current Practice and Epidemiology

Aneuploidy refers to a change in the number of chromosomes present inside a cell from the normal 22 pairs of autosomes plus two sex chromosomes (46 in total), usually caused by incorrect division of chromosomes during gamete formation. Although relatively common in developing embryos, most aneuploidies lead to spontaneous miscarriage early in pregnancy; in 1988, it was estimated that chromosomal abnormalities accounted for 1.8 in 1,000 live births [Baird *et al.* 1998]. Most of aneuploidies present with symptoms including physical abnormalities, sterility and learning disability caused by an increased gene dosage as a result of gaining a particular chromosome. The clinical manifestation of only partial chromosome duplication is extremely variable and may be relatively minor; milder symptoms are also associated with mosaicism, which occurs in around 1% of cases, when chromosomes incorrectly divide after fertilisation resulting in a variable mixture of two types of cells in the body, one with the correct number of chromosomes and the other with a gain or loss of a chromosome.

Currently in the UK, prenatal testing for chromosomal abnormalities is conducted in two steps: screening and risk assessment, followed by prenatal diagnosis of high risk cases. The current gold standard for diagnosis of any aneuploidy is invasive removal of fetal cellular DNA followed by direct examination of the number of chromosomes present (karyotyping). NIPD diagnosis is therefore desirable either if it is able to increase the accuracy of the screening risk assessment, or if it sufficiently robust to reduce or replace the need for amniocentesis/ CVS.

By far the most common aneuploidy compatible with life is Down's Syndrome (DS), or trisomy 21, in which the fetus inherits an extra copy of chromosome 21. DS is associated with variable intellectual impairment, learning difficulties and excess mortality caused by long term health problems such as heart disease. The incidence of DS varies with maternal age, from around 0.07% at age 20 to 1% at age 40 [National Screening Committee 2006]. Uptake for screening is around 70%, for which the tests are typically carried out by hospital biochemistry laboratories; uptake for diagnosis is around 80%, for which tests are generally carried out by cytogenetics laboratories. In 2006, 1,877 new cases of DS were diagnosed in England and Wales, of which 60% were diagnosed prenatally; around 70-90% of these prenatal diagnoses resulted in termination [National Down Syndrome Cytogenetic Register 2006]. It is likely that over 200 healthy fetuses are lost *per annum* due to miscarriage following invasive testing offered as a result of screening.

The current screening protocol for DS comprises several stages, involving blood tests for numerous maternal protein markers and a nuchal translucency (NT) ultrasound scan of the fetus. The results from these tests are combined to calculate a risk score for each pregnancy, which is heavily influenced by maternal age and only reaches its maximum detection rate by integration of multiple different tests. If the risk is greater than 1 in 250, then invasive diagnostic testing is offered. Details of the different screening protocols are outlined in Table 5-1. Current NICE antenatal care guidance recommends using the first trimester combined test if possible, or the second trimester triple or quadruple tests otherwise. In practice, however, first trimester screening only occurs in around 10-15% of pregnancies, and the second trimester triple test is most common, accounting for more than half of those screened, although there is increasing implementation of the combined test in the UK following recommendations from the National Screening Committee. Of the cases of DS that were diagnosed prenatally during 2006, less than a third were diagnosed before 13 weeks [National Down Syndrome Cytogenetic Register 2006], but this may change with more widespread use of the combined test.

Table 5-1 Current screening options for DS
[See Wald *et al.* 2003 for detailed references]

Maternal age with:	Detection rate for 5% false positive rate (%)*	Biomarker (see glossary for biomarker acronyms)
<i>FIRST TRIMESTER (11-13 weeks)</i>		
Ultrasound scan	60	NT
Serum test	74	β -hCG, PAPP-A
Combined test	83	NT, β -hCG, PAPP-A
<i>SECOND TRIMESTER (14-20 weeks)</i>		
Double test	71	free β -hCG, AFP
Triple test	77	AFP, uE ₃ , β -hCG
Quadruple test	83	AFP, uE ₃ , β -hCG, inhibin-A
<i>BOTH TRIMESTERS</i>		
Serum integrated test	90	PAPP-A, AFP, uE ₃ , β -hCG, inhibin-A
Integrated test	93	NT, PAPP-A, AFP, uE ₃ , β -hCG, inhibin-A

* Detection rate (sensitivity) and false positive rate (1-specificity) are related, such that they are both changed simultaneously by altering the cut-off point of the biomarker measurement.

Other aneuploidies with known clinical significance include Edward's Syndrome (trisomy 18) and Patau's Syndrome (trisomy 13), both of which are usually fatal prenatally or within the first few weeks of life. The UK Fetal Anomaly Screening Programme primarily uses ultrasound (NT in the first trimester, or the detailed fetal anomaly scan at 18-20 weeks) to screen for these syndromes. In 2006, 461 cases of Edward and 204 cases of Patau were diagnosed in England and Wales, nearly 90% prenatally, of which more than two-thirds were subsequently terminated [National Down Syndrome Cytogenetic Register 2006].

Aneuploidy of the other autosomes is very rarely seen, but abnormalities associated with the number of sex chromosomes are observed (though not formally included in the screening programme) with highly variable phenotypes, including Turner syndrome (monosomy X), triple X syndrome (XXX), Klinefelter syndrome (XXY) and XYY syndrome.

Current UK policy on working standards for DS screening [National Screening Committee 2007] states that for pregnancies determined to be at high risk of Down's Syndrome, rapid QF-PCR alone should be offered where the ultrasound scan is normal, whilst QF-PCR and karyotyping should be used where the ultrasound scan suggests the presence of a fetal anomaly. Importantly, QF-PCR is a closed-ended test that is currently used following screening to diagnose Down, Patau and Edward's Syndromes only, whilst karyotyping is an open-ended test that could potentially reveal numerous other chromosomal aneuploidies and abnormalities.

5.1.2 Status of cffNA testing

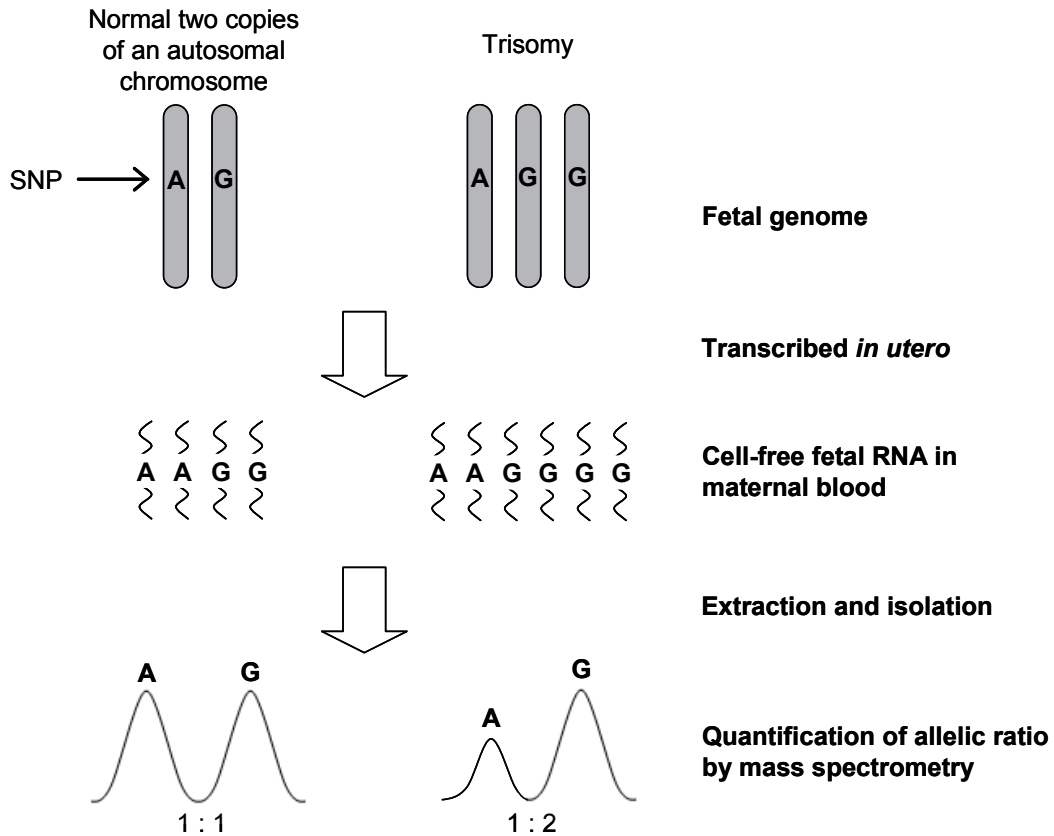
The key distinguishing factor that groups the aneuploidies together is an increase (or decrease) in the relative amount of specific chromosomal material. For the purposes of prenatal diagnosis, detection of aneuploidy therefore requires not just detection but also accurate quantification of the DNA derived from a specific chromosome. However, simple determination of cffDNA concentration has proved to be too inaccurate to use for diagnosis of aneuploidy, due to variation between individuals. Therefore, a number of alternative methods have been developed and tested in small studies, but to date there is no consensus on which method will prove to be the best, although the field is moving extremely rapidly.

Broadly, there are currently two methodologies. The first method exploits the presence of heterozygous SNPs located on the chromosome of interest to calculate a ratio between the SNPs, and thus a ratio between paternally and maternally inherited chromosomes; the ratio is 1:1 in a normal fetus, and 2:1 in a fetus with complete trisomy (see Figure 5-1). The major limitation of this method is its reliance upon the fetus inheriting a conveniently located paternal SNP not present in the mother, the likelihood of which will vary with ethnicity for each individual SNP. Moreover, because of the relatively small proportion of cffDNA in maternal blood, this technique necessitates either selective enrichment or selective detection of fetal genetic elements. To date, this has been achieved by: selective suppression of maternal DNA using formaldehyde⁵; exploiting epigenetic differences in DNA methylation between fetal and maternal DNA; or detecting uniquely placentally-derived RNA from the chromosome of interest, such as mRNA from the *PLAC4* gene located on chromosome 21 [Lo *et al.* 2007a]. This latter method is perhaps the most promising, and is currently under development in the US by the company Sequenom, which is using mass spectrometry to detect multiple SNPs across several placentally-expressed genes. In a press release on 23 September 2008, it announced that the test for DS was expected to cover over 95% of the US population⁶ and had achieved 100% accuracy in over 600 clinical samples to date. A multisite 5,000 laboratory sample test validation study has been initiated, in addition to a multicentre 10,000 person prospective trial, and the test is expected to launch in June 2009.

⁵ A method which has only been reported from one laboratory; other investigators have failed to confirm the results.

⁶ The remaining proportion of the population is homozygous at all the measured SNPs, i.e. have the same sequence, and therefore the method will not work. The test will produce a 'no call' for these individuals, rather than a false result. The population coverage of this test will be significantly reduced in ethnic populations with lower genetic diversity, and therefore a higher proportion of homozygous individuals.

Figure 5-1 Principle of the prenatal determination of fetal aneuploidy by measurement of the allele ratio of a conveniently located heterozygous SNP (for example, within cffRNA from a uniquely placentally expressed gene, as shown below).



The second method measures the chromosome dosage directly by using highly sensitive techniques to compare the ratio of the chromosome of interest to a reference chromosome; the ratio is 1:1 (for most chromosomes) in a normal fetus, and 2:3 in an aneuploid fetus. The major advantage of this method is that it does not rely upon identifying and detecting genetic polymorphisms in the fetus, and therefore should be universally applicable to all aneuploidies in all populations. Techniques include digital PCR, in which enriched single template DNA or RNA molecules are isolated by dilution and then amplified [Lo *et al.* 2007b], and direct high-throughput shotgun sequencing, in which millions of sequence tags are detected across the genome [Fan *et al.* 2008]. Preliminary results from both these techniques suggest extremely high accuracy may be achievable, although the numbers reported to date have been very small.

Whilst most research has focused on DS, either of these methods could in principle be applied to prenatal detection of any trisomy, and proof of principle has already been demonstrated in several cases of Edward and Patau's Syndromes.

In contrast to current approaches to non-invasive screening for DS, cffNA tests have the potential to be much more accurate and potentially even diagnostic, rather than simply predictive of risk. The results to date also suggest that one of the major advantages of using cffNA testing for DS screening would be a decrease in the number of false positive results, and a resultant reduction in the number of invasive diagnostic tests and healthy fetuses miscarried. However, it is currently unclear how cffNA technology might be used in practice for prenatal testing of aneuploidies, as there are broadly five different possibilities:

- (1) As an additional test during screening, to improve the overall risk calculation;
- (2) As an intermediate test in between screening and invasive diagnosis for high risk individuals, to reduce the number of invasive tests requested;
- (3) As a replacement for the current biochemical screening tests;
- (4) As a replacement for invasive diagnostic testing;
- (5) As a replacement for both the current screening tests and invasive diagnostic testing.

Whether cffNA testing will completely replace invasive testing for aneuploidies must await the results of larger scale evaluation to determine its accuracy. Furthermore, the technique may not be applicable to mosaicism and other chromosomal rearrangements. Ultimately, which of these options is used will depend on numerous factors, including technical accuracy, proven clinical performance, feasibility of delivery, service provision and cost effectiveness. (Note that Sequenom is currently planning to position its test as a single screening test, *i.e.* the third option above.)

5.2 Ethical, legal and social issues

One of the major issues in this area is the extent to which distinguishing between screening and diagnosis implies differing legal obligations (for example, in terms of processes or consent) and ethical justifications. For example, literature on screening interventions tends to refer to informed *choice* rather than informed *consent*, and emphasises the importance of taking account of the decision-maker's beliefs and values. Less emphasis is placed upon ensuring competence or freedom from coercion. In the case of DS testing, the impact of this technology is likely to depend in part upon whether it is implemented in addition to existing screening tests, or made available to all pregnant women as a single diagnostic test early in pregnancy. If it were to be introduced universally, there are several important differences between the current screening programme and this proposed use of cffNA testing which are particularly relevant to consider:

- (1) **Multistep versus single test** - the current screening programme is a multistep process in which the patient goes through successive tests, whilst in contrast, cffNA technology could be implemented as a single test. In the absence of this funnelling process, there would be less time for the patient to comprehend and accept the situation, raising communication and support issues.
- (2) **Stratification versus diagnosis** - the current screening programme is used for the purpose of risk stratification, to assess the risk of the fetus having DS, whilst the cffNA test could potentially be used for the purpose of diagnosis.
- (3) **Decision to test versus terminate** - at the end of the current screening programme, the woman faces a decision as to whether to have an invasive test, whereas it is possible that (depending upon its accuracy) patients might choose whether to terminate the pregnancy based only on the cffNA test result, especially in cases where this is available significantly before invasive testing is possible.

- (4) **Earlier diagnosis** - a considerable number of DS fetuses could be diagnosed early in pregnancy that would otherwise have miscarried spontaneously prior to (invasive) diagnosis. This could increase the number of women facing a decision regarding termination, as well as increasing the total number of terminations due to DS.

If cffNA tests were made available to all pregnant women early in pregnancy as a replacement technology, there would be a need to move towards the rigorous informed consent model commonly used for diagnostic testing, where an active decision is made following discussion with a health care professional. Regardless of the model used, in the case of antenatal testing the woman should be fully informed about the possible outcomes of the test and the available options, as underlined by current guidance from the National Screening Committee that *'all women should have a broad understanding of the purpose, risks, benefits, limitations and consequences of fetal anomaly screening'* .

Recent NICE guidance on antenatal care requires that *'pregnant women should be informed about the purpose of any test before it is performed. The healthcare professional should ensure that the woman has understood this information and has sufficient time to make an informed decision. The right of a woman to accept or decline a test should be made clear'*. Information about the condition screened for should be balanced and accurate [NICE 2008]. More explicitly, in relation to Down's Syndrome screening, the guidance requires that information should include the screen-positive and screen-negative pathways, decision points, and *'the fact that screening does not provide a definitive diagnosis'*. Despite being conceptually easier, the potential replacement of the existing series of probabilistic steps with a single diagnostic intervention might nonetheless require a longer and more extensive discussion and arguably more time for the woman to reflect on her choice.

With regards to the method based on cffRNA, the requirement for informative and heterozygous SNPs means that there may be differences in the applicability of the technology to different ethnic groups. Less ethnically diverse populations have less genetic variation and therefore are likely to have fewer heterozygous SNPs. This could potentially lead to issues relating to equity of access, which could be overcome by the addition of more or different SNPs to the test.

As discussed previously, the risk free nature of the technology suggests that its use might be extended to screening for conditions for which prenatal tests are not currently routinely available. This might include screening for certain sex chromosome aneuploidies, such as Klinefelter syndrome (XXY), which are not currently specifically screened for prenatally where molecular techniques (QF-PCR) have replaced karyotyping. The Abortion Act limits the right of a woman to request a termination of pregnancy after 24 weeks *'on the grounds of fetal abnormality'* to serious abnormalities; if more minor conditions are diagnosed prenatally and reported to parents, then if a woman feels that she wishes to terminate the pregnancy as a result, she could only obtain an abortion on social grounds prior to 24 weeks. Changing practice would directly affect women who have diagnoses of minor abnormalities made after 24 weeks gestation, when termination on social grounds is no longer available.

5.3 Perspectives

5.3.1 Patients

There is little question that NIPD would be popular amongst patients as a replacement for invasive testing, as it poses no direct risk to the fetus or mother. Of the calls received by a helpline run by the UK charity Antenatal Results and Choices (ARC), half relate to concerns over invasive testing following Down's Syndrome screening, indicating that there is likely to be a demand for private services if available for DS testing.

Relative to invasive diagnostic testing, blood tests are already commonplace and form an important and accepted part of standard antenatal and primary care. Consequently, there is a danger of routinisation if cffNA testing were offered automatically to all pregnant women, which could result in a reduced emphasis on informed consent and patient autonomy [Van den Heuvel *et al.* 2008]. There is therefore a significant need for education of the public about cffNA technology, including its applications and limitations, in order to increase awareness and manage expectations. There will also be an increased need for support, particularly if the current DS screening process were replaced by a single diagnostic test. It is important to note that some women will not wish to be tested, as they would not be willing to consider termination for cultural, religious or personal reasons, and have a right to refuse any testing.

5.3.2 Physicians

Education of health care professionals, particularly midwives and GPs, is absolutely critical if cffNA tests were to be used routinely in antenatal care. Awareness of the technique, including its application and limitations, is paramount for providing good patient care and is currently very low amongst this group of health care professionals.

Midwives are the primary contact for most women undergoing the current DS screening programme. If cffNA tests were to be offered routinely, it would be important to ensure that the booking appointment was scheduled as early as possible, in order to benefit from early diagnosis. Understanding the exact purpose of the test - as a replacement or addition to the current screening protocol, or as a replacement or alternative to invasive diagnostic testing - would be critical to managing patient expectations and offering appropriate counselling. The accuracy of the test, and the consequences and available options of having a positive result, would also need to be well established and communicated.

5.3.3 Laboratories

The logistics of offering a genetic service at a population level, and the level of laboratory service provision required, are potentially major barriers to implementation of cffNA tests nationally. Current specialist molecular genetics laboratories are not set up to handle such a high throughput, whilst current specialist screening laboratories are not set up to do genetic analysis. Therefore, significant planning and investment into the infrastructure of the laboratory services would be critical prior to offering cffNA tests as part of routine antenatal care. This would include capital costs (for equipment purchase, *etc.*), staff training, quality assurance procedures, appropriate IT provision, and consideration of logistics to ensure equity of access throughout England and Wales. It is currently unclear how many specialist centres would be required, or what level of centralisation would be desirable. Successful implementation of cffNA screening for DS would also have a significant impact on personnel configuration and manpower requirements for conventional cytogenetics. The requirement for prenatal karyotyping may decrease dramatically, but would not be replaced entirely, requiring careful strategic planning for the positioning, deployment and re-training of cytogeneticists for future service delivery models.

Moreover, although QF-PCR is already a relatively commonplace technique throughout the UK, higher throughput and more specialist technologies such as mass-spectroscopy and digital PCR are not yet currently widespread in UK laboratories and might therefore require significant capital investment. Whilst there is a general trend to move towards these molecular techniques, which would most likely have multiple applications in a clinical laboratory, this trend will not necessarily ensure rapidity of service or equity of access.

If cffRNA were to be used for DS testing, the blood sample may need to be centrifuged shortly after being taken, in order to ensure that the mRNA (which is usually much less stable than DNA) is preserved prior to being transported to a laboratory for testing. This requirement could be critical to achieving high test accuracy, and would be extremely difficult to achieve in practice. Currently, phlebotomists have neither the time nor the equipment to spin blood samples immediately after taking them.

5.3.4 Commissioners

The cost associated with the application of cffNA for prenatal testing for DS (and other aneuploidies) is currently unclear, as it is extremely complex and will depend upon the nature of the test itself as well as how it is implemented. It is likely that the availability of cffNA testing would significantly reduce the number of invasive tests required, the majority of which are currently performed in unaffected pregnancies, which would result in a cost saving. However, the total number of women tested for cffNA could be much higher (perhaps 80-90% of all pregnancies) than the number who currently undergo invasive testing (less than 5% of pregnancies). Although the cost of ultrasound screening is relatively expensive, it is unlikely that ultrasound would be abandoned as a result of cffNA testing, due to its applicability to screening for numerous developmental disorders; moreover, the cost of the current biochemical screening tests - around £15-£25 per blood test - is significantly lower than the expected cost of cffNA testing. Therefore, if cffNA testing were to be offered to every pregnant woman, the potential savings from fewer invasive diagnostic tests in high risk individuals could be outweighed by the extra costs of population testing; however, since the test would necessarily be developed on a high-throughput platform, this is too early to judge. A full economic appraisal will therefore be needed once the most appropriate technology (*e.g.* cffDNA versus cffRNA) has been determined.

Before any new test would be considered for use in a national population screening programme, it would be necessary to ensure that it fulfilled the formal screening criteria (see www.nsc.nhs.uk/pdfs/criteria.pdf). A large evidence base would be required, including information on clinical test performance (specificity, and sensitivity), population need, cost effectiveness, models of service, improvement in quality and outcomes, sustainable developments, value for money, and patient acceptability. Within the NHS, a number of strategies must be maintained in order to ensure quality of care, including regular audit and evaluation, a process to ensure equitable provision in designated providers and the provision of this service as part of an integrated service framework. Clear aims about developing this technology for a screening programme need to be identified.

A number of national bodies would be involved in assessing the technology prior to offering it at a population level, including:

- The National Screening Committee (NSC), which would ultimately be responsible for approving and implementing any national screening programmes. Using research evidence, pilot programmes and economic evaluation, it assesses the evidence for programmes against a set of internationally recognised criteria.
- The National Institute of Clinical Excellence (NICE), which is involved in health technology assessments and issuing clinical guidelines about diagnostic tests and patient management. For example, in 2008, NICE released guidance regarding management of pregnancy in RhD negative women.
- The Health Technology Assessment (HTA) Programme, run by the UK National Institute for Health Research, produces independent research information about the effectiveness, costs and broader impact of health care treatments and tests for those who plan, provide or receive care in the NHS. Broadly, the assessment considers whether the technology works, for whom and at what cost, and how it compares with alternatives.
- The Human Genetics Commission (HGC) is the government's advisory body on new developments in human genetics and how they impact on individual lives, with a particular emphasis on ethical, social and legal issues relating to genetic technologies.

6 Management of pregnancy

Many of the routine tests undertaken as part of standard antenatal care are for the purpose of monitoring the health of the mother and fetus, and minimising the risk of complications occurring during pregnancy or at birth. These tests are primarily aimed at informing the physician, rather than the parent, in order to improve the management of the pregnancy, and therefore are generally safe and non-invasive.

Only one specific application of cffDNA technology in this area will be considered in this report, namely the determination of specific fetal blood groups (particularly RhD) in cases of potential incompatibility.

6.1 RhD

6.1.1 Current Practice and Epidemiology

The Rhesus blood group system usually refers primarily to the five main Rh antigens (C, c, D, E and e) found on the surface of red blood cells, of which the RhD antigen is by far the most important. If fetal cells carrying paternally inherited RhD antigens enter the maternal circulation as a result of fetal-maternal bleeding (e.g. due to amniocentesis or during birth), a RhD negative mother may become sensitised and produce an immune response against the fetus in around 13% of at-risk pregnancies [Pilgrim *et al.* 2007]. These maternal antibodies can pass through the placenta and initiate the destruction of RhD positive red blood cells causing haemolytic disease of the newborn (HDN) in more than half of the sensitised pregnancies, a potentially fatal disease characterised by anaemia. This is not usually a problem during the first pregnancy, as very few intact fetal blood cells are exchanged with the maternal circulation. However, at birth there is a significant exchange of blood, often resulting in the production of antibodies which can persist and cross the placenta in later pregnancies, worsening the chance of severe haemolytic disease with each successive RhD positive pregnancy [Pilgrim *et al.* 2007].

Whether a person is RhD positive or negative is determined genetically by the dominant *RHD* gene. The majority of RhD negative individuals are Caucasian, in whom the *RHD* gene is completely deleted; in other ethnic populations (such as Asians, Japanese and black Africans) the RhD negative phenotype is less common and generally associated with numerous genetic variations including point mutations and small DNA insertions in the gene. The RhD antigen is present in around 85% of the general population in the UK (although this figure varies across ethnic groups and is present in around 99% of people of African origin) and accounts for the majority of cases of maternal immunisation.

Currently, this immune response is treated prophylactically by offering all RhD negative pregnant women an injection of anti-D antibodies (which bind to and neutralise fetal RhD antigens) regardless of the RhD status of the fetus. This injection is typically administered after any risk prone incident (such as amniocentesis or CVS), during the third trimester (28 and 34 weeks) and immediately following birth. Prior to the introduction of anti-D prophylaxis, HDN due to RhD incompatibility affected around 5% of children born to RhD negative women in Caucasian populations; currently, the figure is closer to 0.5%. However, it has been estimated that in the Caucasian population, 40% of the RhD negative women receive unnecessary antenatal anti-D whilst carrying a RhD negative child. Of the 700,000 pregnancies in the UK annually, around 15% occur in RhD negative women; therefore, around 40,000 women currently receive unnecessary prophylaxis.

Minimising the prophylactic use of anti-D may be desirable, as it is relatively expensive and could theoretically transmit infection since it is harvested from blood donors. Although determination of fetal RhD status can be achieved by amniocentesis or CVS, both these methods carry a risk of not only miscarriage but also of maternal sensitisation to RhD due to mixing of fetal and maternal blood. Invasive testing is therefore reserved only for women who are already sensitised, and are therefore already at high risk of HDN, and even then it is not generally recommended.

Other rarer blood cell antigens can also cause haemolytic disease of the newborn in mothers who are negative for the corresponding antigen, in a manner analogous to RhD maternal immunity. The presence of these antigens in the fetus could also be determined prenatally using cffDNA, again reducing the unnecessary use of prophylactic antibodies.

6.1.2 Status of cffNA testing

Since the *RHD* gene is deleted in the majority of RhD negative Caucasians, a paternally derived fetal *RHD* gene can be specifically amplified and detected with high levels of accuracy using real-time QF-PCR. Problematic false negatives are generally attributed to failure to detect the presence of fetal DNA, which could be addressed by concurrently utilising other methods for universal fetal DNA detection as an internal control; false positives are less critical clinically, as the administration of unnecessary anti-D therapy is perceived as a low risk intervention, and has been the mainstay of management of this condition. In some laboratories, fetal sex determination using *SRY* is simultaneously performed to ensure that the assay is working correctly; however, this method only works in male pregnancies and inclusion of a universal fetal marker would clearly be preferable, and some researchers include biallelic STR sequences for this purpose.

Numerous and extensive studies have been published regarding the accuracy of determination of fetal RhD status from cffDNA, a selection of which are summarised in Table 6-1 [adapted from Van der Schoot *et al.* 2008]. A meta-analysis of 37 studies published between 1993 and 2005 found that using cffDNA from maternal plasma produced an overall diagnostic accuracy of 96.5%, with the highest diagnostic accuracy being in the first trimester [Geifman-Holzman *et al.* 2006]; more recent studies have shown a substantially improved accuracy. In addition to testing for the RhD antigen, cffDNA in maternal plasma can also be used to diagnose the presence of other rare blood cell antigens K (Kell), Rh C, c and E as well as ABO blood group in mothers whose blood cells lack the respective antigen [Finning *et al.* 2007].

CffDNA testing is currently used routinely in the UK for women at high risk of HDN, including previously RhD sensitised women and those where a rising level of antibodies is detected. The International Blood Group Reference Laboratory (IBGRL) in Bristol has been offering this service since 2001, and currently tests around 200 women *per annum* at 26-32 weeks, with no discordant results since 2005 [Geoff Daniels, personal communication]. If NIPD were to be offered to all RhD negative pregnant women in the UK (see Figure 6-1 for proposal), around 100,000 tests would be required annually. In addition, testing would need to occur before 28 weeks, when the first dose of anti-D is offered, which will require validation in large trials to determine the diagnostic accuracy of high-throughput techniques. Using the published false positive rate of 0.2% achieved by the IBGRL at 28 weeks [Finning *et al.* 2008], and assuming countrywide coverage, this would put around 8 pregnancies *per annum* at risk of HDN, potentially resulting in a single sensitised pregnancy each year.

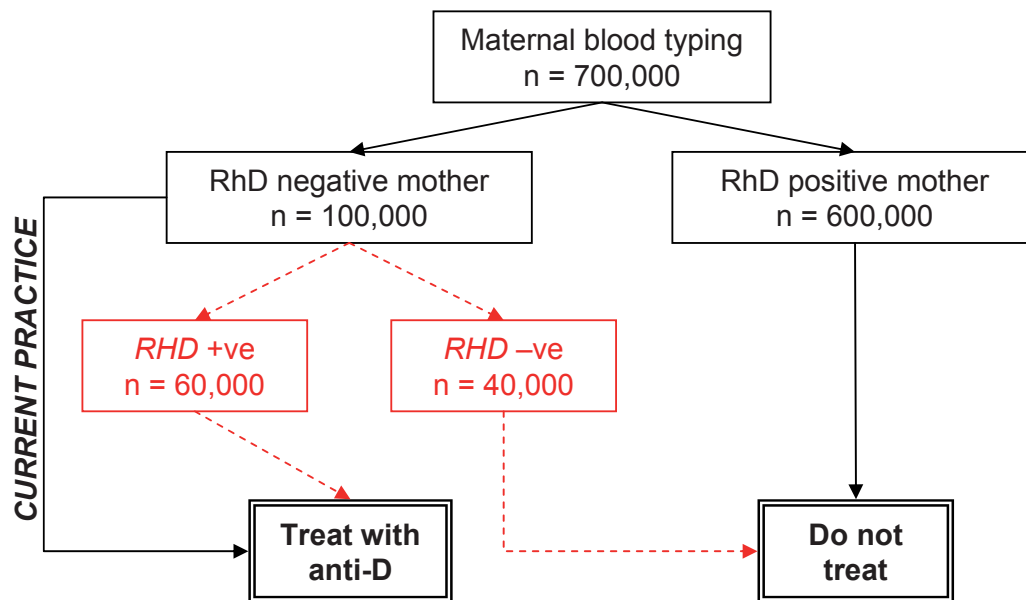
Table 6-1 Representative selection of recent studies using real-time QF-PCR for non-invasive prenatal diagnosis of fetal *RHD*.

Author (Date)	Country	N	Gestation (weeks)	Sensitivity (%)	Specificity (%)
Rijinders (2004)	Holland	72	11-19	100	96.6
Brojer (2005)	Poland	230	5-39	100	98.6
Gautier (2005)	France	283	8-35	100	100
Rouillac-Le Sciellour (2007)	France	300	10-34	100	97.5
Minon (2008)	Belgium	563	10-38	100	99.5*
Finning (2008)**	UK	1997	8-38	99.8	99.2

* 1 false positive from recipient of a RhD positive organ transplant

** International Blood Group Reference Laboratory (Bristol, UK); includes a test for the most common African *RHD* variant, which accounts for 66% of phenotypically RhD negative black Africans; most samples were tested at 26-32 weeks gestation.

Figure 6-1 Flow diagram outlining process for RhD testing from maternal blood, including approximate UK numbers for all potential pregnancies



Key:

Current practice in black; addition of fetal genotyping in red.

6.2 Ethical, legal and social issues

Many of the general ethical, legal and social issues raised by NIPD do not apply to fetal RhD testing, which would be used for the purposes of determining the best way to manage the pregnancy to minimise the health risks to the mother and fetus. There is, however, considerable concern about false negative results, where the mother would not be given anti-D and therefore might become sensitised. Since this risk is preventable under the current model, there remains a dilemma regarding the trade-off between cost, risk prevention and quality of care.

The possibility of inadvertently revealing non-paternity is a theoretical concern in this area, *i.e.* in cases where both the mother and the assumed father are RhD negative but the fetus is found to be positive. Since the test could potentially be offered to all RhD negative women during pregnancy (approximately 100,000 *per annum*), the potential for finding non-paternity is high, although a definitive finding of non-paternity is only likely to arise if the putative father's RhD status is already known. (The explicit testing of paternal blood is unlikely to occur outside the clinical genetics setting where there is a clinical justification for testing, such as the need to establish whether the father is a carrier of a recessive mutation). Given that tests for RhD status are likely to be determined along with other routine blood tests, it is plausible that the potential consequences of the test will not be fully understood by, or explained to, the woman. Whilst it is debatable whether it is the responsibility of the health professional providing screening to warn of the theoretical likelihood of non-paternity being revealed as part of the process of obtaining consent (particularly if making an inference of non-paternity will require knowledge outside the clinical environment), health professionals will need to understand the theoretical risks involved.

6.3 Perspectives

6.3.1 Patients

It is unclear whether NIPD would be a popular choice for RhD testing, where anti-D therapy is not generally perceived to be undesirable by women and has a very limited risk associated. However, some women do not want to take anti-D because it is a human blood product.

6.3.2 Physicians

The potential impact of a test for fetal RhD status is dependent upon whether the results of the test change the treatment and care pathway, *i.e.* would women with a negative result still be offered anti-D therapy? Significant education within haematological services as well as maternity services would be needed if testing were to be offered to all RhD negative pregnant women. Health care professionals would also need to be made aware of the possibility of inadvertently revealing non-paternity, which is not an issue that haematology units are commonly accustomed to dealing with.

Whilst testing would be able to fit into the current antenatal care pathway, as the only requirement is that the test results are available before the first dose of anti-D is given (28 weeks), it is not clear yet which appointment would be best in terms of logistics and technical accuracy. The workflow and health records would need to be integrated, to ensure that results from maternal blood typing were received prior to sending or analysing (only) RhD negative blood for fetal RhD genotyping. Results of fetal RhD would then need to be checked before 28 weeks, when the first dose of anti-D is usually administered, which is a somewhat more complex care pathway than simply giving anti-D to all RhD negative women, and might involve splitting blood samples to send to different laboratories.

6.3.3 Laboratories

Fetal RhD diagnosis for high risk (sensitised) women would not require any laboratory changes, as this service is already currently offered nationally by the Bristol International Blood Group Reference Laboratory. However, if test provision were to be extended to all RhD negative women (~100,000), investment in laboratory infrastructure would probably be needed to support high-throughput testing. Currently, haematology laboratories are not set-up to do genetic testing, but molecular genetics laboratories, whilst well equipped, are geared to significantly lower throughputs.

6.3.4 Commissioners

The use of cffDNA to determine the RhD status of the fetus in RhD negative women could potentially reduce the number of women to whom anti-D prophylaxis is administered by as many as 40,000 pregnancies *per annum* in the UK. This equates to a saving of around £1.6 million *per annum* in anti-D⁷. However, although few doubt the clinical and cost benefits of testing high-risk sensitised women, the cost-effectiveness of testing all RhD negative pregnant women remains controversial; given the cost of testing around 100,000 women, it is possible that this application could increase the overall cost of antenatal care for RhD negative women. A full economic assessment, including a detailed cost/benefit analysis, will be required before routine antenatal testing of fetal RhD status is recommended by either the NSC or NICE. Given that current care does not seek to distinguish between RhD positive and negative outcomes, commissioners will need to be persuaded that there are good medical or economic reasons to implement the test.

In August 2008, NICE released guidance on the clinical management of pregnancies in RhD negative women, recommending the use of anti-D prophylaxis routinely. Although the use of cffDNA tests to determine fetal RhD status was considered, it was thought that the technology was not yet accurate enough to be beneficial for this application. At the next revision of this guidance (in May 2011), the level of accuracy that NICE would require to include the use of this test in guidance will be informed by a technology assessment. The NSC Policy regarding the use of cffDNA tests for fetal RhD will also be reviewed in 2009-10.

⁷ Of the ~700,000 pregnancies per annum in England and Wales, ~15% are in RhD negative women, of whom ~40% are carrying a RhD negative child and currently receive anti-D unnecessarily at a cost of ~£40 each [Pilgrim *et al.* 2007].

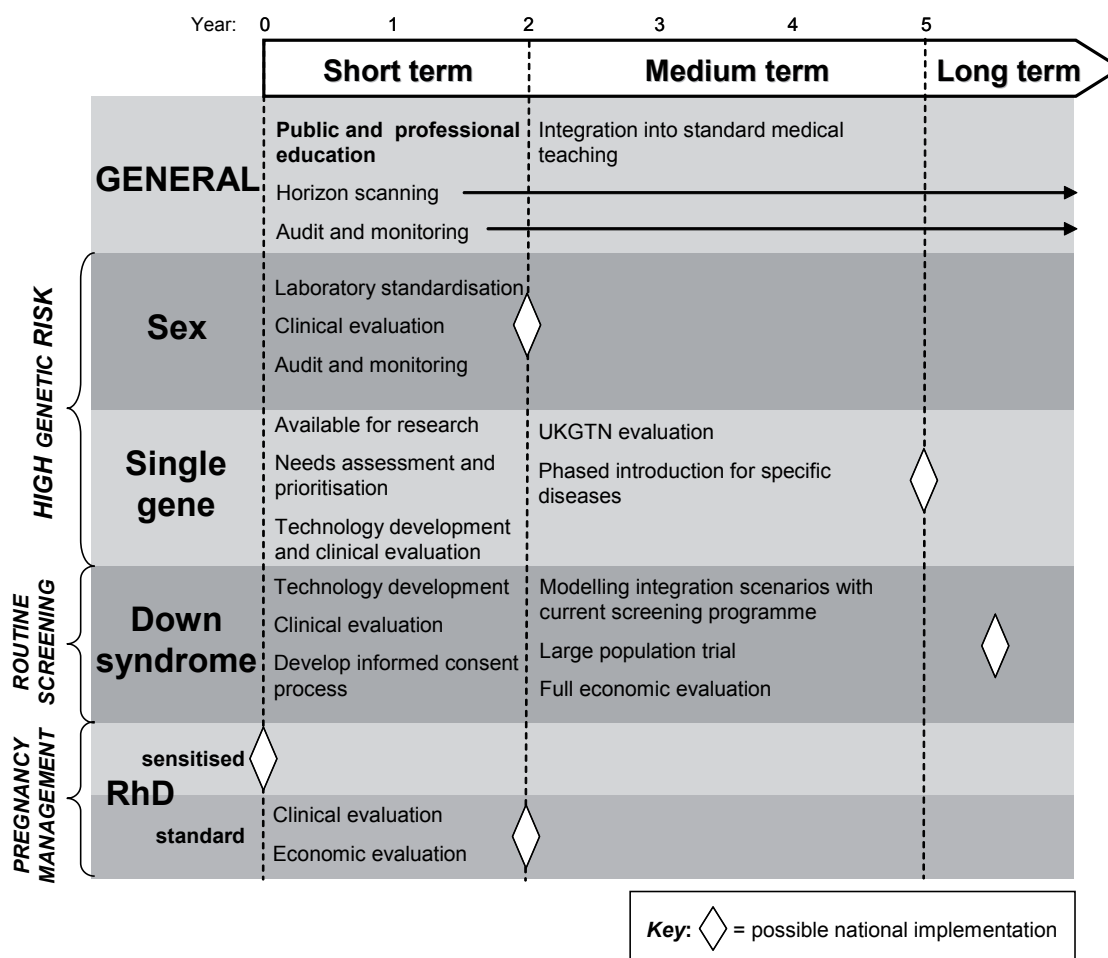
7 Findings and Recommendations

7.1 Key findings

The working group believes that the implementation of non-invasive prenatal diagnosis for clinically significant genetic disorders is desirable, both to improve the quality and management of antenatal care and to facilitate parental reproductive choice, and that the development of cell-free fetal nucleic acid technology for these purposes should be supported within the UK.

However, careful consideration of each of the application areas of cffNA testing, and management of the process of implementation within the NHS, is required to ensure effectiveness, quality, value and equity of service. We therefore highlight the following key areas that need to be addressed before this technology is implemented into the NHS, and make recommendations with a suggested timeline for action and implementation for each application (see Figure 7-1). In order to facilitate policy development in this area, the key findings from this work were submitted as evidence to the House of Lords Sub-Committee on Genomic Medicine in October 2008 (available at www.phgfoundation.org/file/4384).

Figure 7-1 Recommended timeline for action and likely timeline for implementation of NIPD across the NHS



7.2 Recommendations

7.2.1 Timeline for Implementation

NIPD using cell-free fetal DNA (and RNA) is likely to become increasingly available within the next 3-5 years, and the NHS should take steps now to ensure that it is able to respond in a timely and appropriate manner as the technology develops.

The use of cffNA for NIPD is a highly dynamic area, and the technology is rapidly changing and improving. This means that the likely timeline for implementation must remain flexible, and the priority for any actions indicated along the timeline may change depending upon the state of the science. It is also expected that implementation of cffNA testing will vary with different applications, depending upon the level of available evidence associated with that application. At this point in time, the best estimate for when implementation of NIPD using cffNA testing will be possible for the different applications is as follows:

Immediate	RhD testing in high-risk sensitised women recognised as standard practice
Within 2 years	Fetal sex determination (for clinical indications); Fetal RhD testing in RhD negative women
Next 3-5 years	Testing for certain single gene disorders; Pilot Down's Syndrome and aneuploidy testing
5+ years	Large validation trials for Down's Syndrome and possible incorporation into screening programmes

7.2.2 Public Engagement

Public engagement is urgently needed and should be pro-actively pursued with a clear, accurate and consistent message that recognises the limitations of cffNA testing.

A reliable and consistent message needs to come from all the relevant parties - including professional bodies, NHS information resources, and patient organisations - to keep the public well informed and updated regarding the development of cffNA technology for NIPD. This is critical for safe-guarding public trust in scientists and the NHS as well as managing public expectations about the technology and providing accurate information on the current status of scientific development. In particular, the prerequisites for, and the process by which such technologies can be implemented into the NHS should be more transparent and details of current research and development activities within the NHS made public.

Public engagement should be pro-actively pursued through multiple organisations and dissemination methods. Resourcing will be required to maintain an ongoing and increasing level of public education as the technology nears universal implementation. However, achieving the critical balance between raising public *awareness* and raising public *expectations* will be difficult, and requires the process to be carefully managed to ensure that the information is evidence-based, consistent, coherent and regularly updated. Several information dissemination strategies are desirable, including:

- regular bulletins from the National Screening Committee;
- statements from the relevant Royal Colleges;
- provision for information on the NHS Direct website;
- development of a new, authoritative website specifically for NIPD, with information provided and moderated by the recipients of the RAPID grant;
- involvement of professional centres with experience in public engagement, such as the Nowgen Centre for Genetics in Healthcare.

7.2.3 Professional Education

Professional education is urgently needed, particularly for key health care workers and should be started immediately by relevant professional bodies.

The introduction of cffNA testing will necessitate a major programme of education amongst numerous groups of health care professionals, including GPs, midwives, obstetricians, fetal medicine experts, clinical geneticists and gynaecological nurses. Moreover, since certain tests are already available on a DTC basis over the internet, women may actively seek NIPD through private providers; health care professionals must therefore be equipped to answer questions about available technologies, test performance, applications and limitations, as well as its current status in order to provide advice.

In order to safeguard public trust in health care professionals, this education programme should be started immediately, both to ensure that members of the public receive effective and accurate advice from their GP, midwife or other antenatal service providers about cffNA testing, and to manage the expectations of health care professionals themselves. In the short-term, this process should be initiated by the National Genetics Education and Development Centre (NGEDC) and relevant professional bodies through formal information channels. In the medium and long-term, professional education about cffNA and its application to NIPD should be integrated into specialty training programmes and ultimately into routine medical teaching.

7.2.4 Evaluation

Formal evaluation of the use of cffNA testing for each application within a specified care pathway should be undertaken prior to implementation within the NHS.

The use of cffNA for NIPD within each application should be fully evaluated prior to implementation across the NHS, in order to establish the technical and clinical accuracy of each test. The ACCE framework should be used as a guideline for this process, which would include determining the analytical validity of each cffNA assay, as well as the clinical validity and utility of each test within a clinical pathway. Although the UKGTN gene dossier system (which is based on the ACCE framework) provides an excellent model, there are a number of areas in which the process needs to be adapted to make it both practicable and appropriate to cffNA tests:

- As part of their validation process, the UKGTN currently requires a new gene dossier for every specific application of a particular technology, which will be inappropriate for validating fetal sex determination for any sex-linked (or endocrine) disorder using cffDNA. Therefore, although the key elements of the evaluation process are still applicable, current UKGTN procedures would not be suitable for the validation of a general cffNA test for fetal sex determination, and an alternative process might be needed.
- The remit of the UKGTN is currently limited to the review of nucleic acid tests for inherited disorders, which are generally rare and offered through highly specialist services to a select group of patients at high risk of a specific disorder. To date, however, the gene dossier process has not been applied to the validation of screening tests that are offered on a population level, for which different and more extensive supporting evidence may be required. An appropriate framework is likely to include the use of small, high risk groups for initial clinical validation of a particular test, followed by pilot studies in large cohorts prior to implementation. We recommend that the UKGTN should work with the NSC to formulate criteria and standards for the evaluation of genetic screening tests.

In the longer term, there is an increasing need for a formal procedure to evaluate all novel diagnostic tests, which would provide bodies such as the NSC with advice regarding the entry criteria for the introduction of a new test.

A full economic assessment and cost/ benefit evaluation also needs to be carried out for each of the clinical applications of cffNA testing, taking into account factors including cost and likely number of tests, changes to current clinical practice, service provision and benefits of the test. This assessment should be done as soon as possible for the two most technically advanced applications - fetal sex determination for pregnancies at risk of a sex-linked disorder and fetal RhD determination in RhD negative women. The most complicated assessment will be for Down's Syndrome, for which multiple models should be considered depending upon the purpose of the test and the mode in which it is offered, *i.e.* in parallel with the current screening programme, or as a replacement for screening and/or diagnostic testing. Any assessment should include an evaluation of the number and timing of terminations of pregnancy sought on grounds of fetal abnormality.

7.2.5 *Audit and monitoring*

Formal audit and monitoring processes should be established for all non-invasive prenatal tests based on cffNA technology.

To date, the effectiveness of cffNA tests has been assessed as part of the research process. However, as the technology becomes integrated into mainstream clinical care, there is an increasing need for a formal audit process to be set up as soon as possible to monitor both the purpose and outcomes of all prenatal tests involving cffNA technology. Although there are challenges in terms of resources and logistics, a formal audit and monitoring process is absolutely essential.

Consideration should be given to the establishment of a case registry for NIPD and prenatal testing generally, which should include data on validated outcomes wherever possible, and could be tied in with other relevant case registries such as congenital anomaly registers (where available). The identity of the data holder, the extent to which any of the information was coded or de-identified and used to inform local health care practice, as well as the need for express consent for data collection should be taken into account. The extent to which data should and could be shared for audit purposes is currently under discussion, and there is a lack of consensus as to the extent to which identifiable patient information can be shared outside the immediate clinical team. Since data will probably be shared across clinical boundaries, express consent from patients may need to be sought - particularly if there is to be follow up of cases. Over time, the register could be used to monitor the extent to which the technology is applied outside current clinical boundaries (specification creep).

7.2.6 *Clinical services*

National best practice guidelines should be developed by key clinical services to ensure that cffNA testing is only used within agreed clinical pathways.

For women at high risk of carrying a fetus with an inherited genetic disorder, cffNA tests should not be offered outside an agreed clinical pathway or without consultation with a clinical geneticist. Work needs to be done to understand which specialties are involved (fetal medicine units, clinical geneticists, *etc.*) and how referral and communication processes should and do work between these specialties. Clinical geneticists should work with fetal medicine units to develop key competences, standards and clinical pathways for professionals offering services and interpreting results, in order to formulate best practice guidelines and ensure appropriate levels of support. In the short-term, these should explicitly include back-up tests and obtaining a placental sample at delivery in order to verify the results obtained through NIPD. Quality assurance frameworks should be developed to extend beyond the laboratory and should include the whole pathway from providing information about the test, through to testing, giving results and subsequent action.

The implementation of cffNA testing within routine antenatal care, either for RhD negative women or for Down's Syndrome testing, necessarily has enormous implications for maternity services. Aside from consideration of how to provide for suitably early and informed decision-making, the logistical and financial impact of a comprehensive move from the current screening programme to a system of non-invasive diagnostic testing would be significant, and will necessitate careful assessment and planning by the NSC. The potential knock-on effect on other services and resources, such as access to termination, would also require careful consideration given the large annual number of pregnancies.

7.2.7 *Laboratory services*

Standard protocols should be developed by expert laboratory services that include the whole care pathway, supplemented by quality assurance frameworks to ensure accuracy, reliability and comparability of results.

Currently, cffDNA testing is only offered by a few specialist laboratories within the UK. It is unclear how laboratory service provision of this technology should be structured in the future, including whether it should be based in laboratories with expertise in a particular disease or the methodology itself. Although it may be inappropriate to restrict it to selected laboratories, developing laboratory competence through training, appropriate resourcing and the use of reference material and standards is critical. Before further expansion of the technique proceeds, a quality assurance framework should be developed, including consideration of the whole patient testing pathway. The limits of gestation for testing must be determined, and standardisation of protocols by laboratories with considerable expertise in cffDNA testing is essential to ensure accuracy, reliability and comparability of results.

In the longer term, technological development will be required to produce platforms and IT systems that can cope with a high throughput of samples, as well as large quantities of data requiring careful analysis. Since it is currently unclear which technology is most likely to prevail for Down's Syndrome testing, recommendations regarding equipment provision, staffing and infrastructure development would be premature. However, specialist molecular genetics laboratories are not currently set up to handle such a high throughput, whilst specialist screening and haematology laboratories are not currently set up to do genetic analyses. Therefore, significant planning and investment into the infrastructure of the laboratory services will be a critical prelude to offering cffNA testing as part of routine antenatal care. It is currently unclear how many specialist centres would be required, or what level of centralisation would be desirable. The NSC should maintain close links with researchers, so that it is well placed to respond quickly as the technology develops.

7.2.8 *Private services*

Private NIPD services are already available on a direct-to-consumer basis, which will impact on NHS services; although the extent to which regulation can and should be applied to these services is debatable, development of a voluntary code of conduct should be supported to help ensure quality.

A formal position regarding the use of cffNA tests for non-clinical applications, such as social sex selection and paternity testing, is outside the remit of this working group. However, it is important that policy-makers recognise that development of the technology for clinical purposes will no doubt also give rise to non-clinical uses, and that the relative ease of taking a blood sample means that (unlike invasive prenatal diagnostic tests) cffNA tests are inherently well suited to the DTC marketplace.

It is likely that both clinical and non-clinical applications will become available DTC, which may result in a significant public pressure on the NHS not only to offer such tests, but also to provide support to those who have sought testing privately. However, private companies are not always explicit about their test performance standards, and hence to what extent the results can be relied upon to be accurate. This working group supports the recommendation of the Human Genetics Commission (HGC) in its call for a voluntary code of conduct and proposal to establish a “Common Framework of Principles”, and urges the UK government to require private test providers to supply evidence of quality assurance.

The role of the regulators in regulating DTC genetic tests more widely is currently being debated and considered by bodies such as the HGC, the Department of Health and the MHRA. Although it is unlikely that the NHS will be able to act as the sole ‘gatekeeper’ to this technology, it has a duty of care to those who seek the tests elsewhere, and should therefore provide support and counselling to those who need it. There may therefore be a ‘knock-on’ effect on NHS services if DTC tests become more widespread.

7.2.9 *Oversight and commissioning*

Oversight from appropriate authorities is needed to ensure responsible, effective and timely implementation of cffNA technology for NIPD within the NHS.

There is a need for oversight of NIPD, in order to ensure responsible, effective and timely implementation of cffNA testing within the NHS. However, since the application areas are so diverse, the responsibility for oversight should be split into two groups:

- (1) The implementation of cffDNA testing for high risk pregnancies (for sex-linked and single gene disorders) should be overseen primarily by GenCAG, with guidance from the UKGTN and other relevant professionals and experts. There needs to be discussion of how the gene dossier system could be adapted, to ensure that the UKGTN can be responsible for the equitable provision of tests.
- (2) The implementation of cffNA testing into routine antenatal care (for RhD and Down’s Syndrome) should be overseen by the NSC, which should hold regular meetings with relevant experts, including the steering committee of the RAPID grant, in order to keep updated on progress.

There is an immediate need for NHS commissioners to recognise the current application of cffDNA testing for prenatal RhD determination in high risk sensitised women, and for them to proceed with formal commissioning processes to ensure appropriate service provision. The test should be integrated into national guidelines and form part of standard care for sensitised women. Additionally, the results from the ongoing trial of early fetal RhD diagnosis in non-sensitised women should be provided to NICE prior to 2011, when the current guidance on routine antenatal anti-D prophylaxis for RhD negative women will be updated.

The NHS National Horizon Scanning Centre (NHSC) should be made aware of cffNA technology and its development, as well as the status of any competing NIPD technologies. The Department of Health should take a lead in evaluating the intellectual property rights in this area and, if necessary, negotiating terms to ensure suitable access and value. However, the NHS should avoid paying license fees whilst it is still actively engaged in research. The NHS should consider the likely market volume and economic impact of each application of the technology in order to negotiate a 'reasonable' arrangement, and whether the arrangements should be negotiated at a national (centralised) or local level for each application. Given what is currently known about the costs of the competing technologies, setting up a collaborative UK-based evaluation study with a commercial partner, as part of the large clinical trials currently ongoing in the US, may be an appropriate way to proceed regarding Down's Syndrome testing. The recipients of the RAPID grant award should liaise with the NSC prior to designing these studies, to ensure that the trials will generate the necessary information to inform the rigorous evaluation process required.

It is important that NHS commissioners work with the UKGTN to ensure equity of access to services as appropriate. In order to get a cffNA test commissioned, a business case will need to be made for each application, which sets out the validity and utility of that test and its influence on current care pathways. In order for the NHS to commission a new test at a population level, commissioners will need to be persuaded that there is an unmet medical need, a significant financial saving or an improvement in the quality of care, which can be effectively addressed using cffNA technology.

7.2.10 Additional research

Additional research is needed to investigate some of the broader implications of cffNA testing for NIPD.

In addition to scientific, technical and clinical development, further research is also needed into a number of broader issues:

- how cffNA testing will affect current maternity services and clinical care pathways;
- what trade-offs women are prepared to accept in terms of test accuracy versus risk to the pregnancy;
- the occurrence and acceptability of specification creep;
- trends in the use of prenatal diagnosis and consequences for pregnancy outcomes;
- the appropriateness of informed consent versus informed choice models within NIPD and antenatal screening programmes;
- the extent to which this type of prenatal testing becomes 'routine' and the effect this might have on informed consent.

8 Conclusion

The use of circulating cell-free fetal nucleic acids (DNA and RNA) for non-invasive prenatal diagnosis is a relatively new technique that has significant potential to improve various forms of antenatal testing within the NHS. At present, this technology is being used in the UK for RhD testing in high risk sensitised women, and in specialist centres for sex determination in pregnancies at risk of specific inherited diseases. Future applications will include screening for Down's Syndrome, testing for single gene disorders and fetal RhD testing in all RhD negative women. Although further evaluation will be needed to determine how effective the tests are for different applications, this rapidly developing field is likely to significantly affect both specialist and routine antenatal care within the next 3-5 years.

This report has been produced by the PHG Foundation in conjunction with an expert Working Group consisting of representative stakeholders, including clinicians (GPs, midwives, obstetricians and geneticists), scientists, NHS commissioners, public health experts, ethicists and patient representatives. We have reviewed the technological development and clinical application of cffNA testing for NIPD, as well as outlining some of the major ethical, social and legal implications of the technology. We have also highlighted some of the key issues that will need to be addressed if the technology is to be implemented within the NHS, and identified the next steps that need to be taken by numerous stakeholders and partners.

Major recommendations include professional and public education, development and implementation of appropriate clinical care pathways, laboratory standardisation and infrastructure development, continuing professional oversight, and formal evaluation coupled with long-term monitoring of prenatal testing. Due to the rapid development of this technology and its potential impact on antenatal care, it warrants close scrutiny over coming months as it progresses. The recommendations outlined here are essential for the successful, timely, effective and appropriate implementation of cffNA testing within the NHS.

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10 Appendices

Appendix I: Working group membership

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Appendix II: Technical review abstract

Wright CF & Burton H. The use of cell-free fetal nucleic acids in maternal blood for non-invasive prenatal diagnosis. Human Reproduction Update (2009); 15:139-151

BACKGROUND: Cell-free fetal nucleic acids (cffNA) can be detected in the maternal circulation during pregnancy, potentially offering an excellent method for early non-invasive prenatal diagnosis (NIPD) of the genetic status of a fetus. Using molecular techniques, fetal DNA and RNA can be detected from 5 weeks gestation and are rapidly cleared from the circulation following birth.

METHODS: We searched PubMed systematically using keywords free fetal DNA and NIPD. Reference lists from relevant papers were also searched to ensure comprehensive coverage of the area.

RESULTS: Cell-free fetal DNA comprises only 3-6% of the total circulating cell-free DNA, therefore diagnoses are primarily limited to those caused by paternally inherited sequences as well as conditions that can be inferred by the unique gene expression patterns in the fetus and placenta. Broadly, the potential applications of this technology fall into two categories: first, high genetic risk families with inheritable monogenic diseases, including sex determination in cases at risk of X-linked diseases and detection of specific paternally inherited single gene disorders; and second, routine antenatal care offered to all pregnant women, including prenatal screening/diagnosis for aneuploidy, particularly Down's Syndrome (DS), and diagnosis of Rhesus factor status in RhD negative women. Already sex determination and Rhesus factor diagnosis are nearing translation into clinical practice for high-risk individuals.

CONCLUSIONS: The analysis of cffNA may allow NIPD for a variety of genetic conditions and may in future form part of national antenatal screening programmes for DS and other common genetic disorders.

Please visit humupd.oxfordjournals.org or email caroline.wright@phgfoundation.org for a copy of this paper.

Appendix III: ELSI review paper abstract

Hall A, Bostanci A & John S. Ethical, legal and social issues arising from cell-free fetal DNA technologies (2008).

This paper provides an analysis of ethical, legal and social issues raised by the introduction of cell-free fetal DNA (cffDNA) technology for non-invasive prenatal testing. We recognise the clinical benefits of cffDNA technology in terms of a reduction of invasive procedures (amniocentesis and CVS) as well as better targeting of pregnancy-related interventions. However, these benefits notwithstanding, the introduction of this technology is not necessarily ethically unproblematic if seen in the context of broader debates about prenatal testing, and if one considers the particular characteristics of cffDNA technology. The paper also examines a range of potential applications for the technology within the context of clinical genetics services, routine antenatal care, and direct-to-consumer testing.

Use of cffDNA technology within existing specialist clinical genetics services provides a valuable tool for earlier and safer determination of fetal sex relevant to inherited (sex-linked) disorders. However, the availability of non-invasive tests using cffDNA could also lead to an increase in prenatal testing and to selective terminations of pregnancy in circumstances currently only possible in the highly regulated context of preimplantation genetic diagnosis (PGD). The ability to test earlier in pregnancy could influence the choices made by parents to continue with the pregnancy and could even make it a more difficult choice to continue with an affected pregnancy. More generalised use of non-invasive testing could facilitate selective terminations of pregnancy in a range of conditions hitherto not diagnosed prenatally and where the arguments for and against termination may not have received sufficiently scrutiny.

In antenatal care, cffDNA technology has already been introduced for the management of RhD incompatibility in high risk sensitised pregnancies. However, test parameters and the appropriate scope for the general application of cffDNA testing in antenatal care remain to be established. With respect to antenatal screening for aneuploidies such as Down's Syndrome, the introduction of cffDNA or cffRNA analysis could replace current screening methods with testing programmes that allow for non-invasive and highly predictive diagnostic testing. Albeit likely to be 3-5 years in the future, the prospect of the introduction of cffDNA technology in routine antenatal care underlines the importance of informed consent in the design and evaluation of antenatal screening programmes.

In the light of these issues, we suggest that it may be useful to consider the introduction of cffDNA technology as part of a more general shift towards non-invasive prenatal testing. One area of potential concern is the application of the technology for fetal sex determination for non-medical reasons, for example by means of tests already available from commercial providers via the internet, with a view to sex-selective abortion. We examine some of the arguments that could be made for and against such uses and review some possible implications for the NHS. More importantly, however, this illustrates that the introduction of cffDNA technology raises questions about the adequacy of existing legal frameworks and about appropriate policy and regulatory interventions that transcend health care providers.

Please visit www.phgfoundation.org or email alison.hall@phgfoundation.org for a copy of this paper.



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