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Horizon Scanning Technology Prioritising Summary

Non-invasive prenatal diagnostic test for trisomy-21 (Down's Syndrome)

Update: November 2009

Adelaide
Health Technology
Assessment

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PRIORITISING SUMMARY (UPDATE 2009)

REGISTER ID: **000303**

NAME OF TECHNOLOGY: **NON-INVASIVE PRENATAL DIAGNOSTIC TEST FOR TRISOMY-21 (DOWN'S SYNDROME)**

PURPOSE AND TARGET GROUP: **FOR THE DETECTION OF FOETUSES WITH TRISOMY-21**

NOVEMBER 2009 – BACKGROUND

A reliable method for the non-invasive prenatal diagnosis (NIPD) of foetal conditions including Down's Syndrome is required to reduce the risk of miscarriage that is associated with chorionic villus sampling or amniocentesis. It is also hoped that NIPD will enable an earlier diagnosis as cell-free foetal RNA and DNA can be detected at five weeks gestation. Chorionic villus sampling and amniocentesis are performed at 11-14 and 15 weeks gestation, respectively. A recent review of the field by Wright and Burton (2009) describes a number of potential clinical applications using cell-free foetal nucleic acids (cffNA) including:

* Sex determination by the detection of cffDNA sequences on the Y chromosome;
* Single gene disorders by the detection of a *paternally* inherited allele in cffDNA;
* Pregnancy-related disorders by the detection of the presence of a working copy of the Rhesus gene (RhD) or an elevation in the absolute concentration of cffDNA; or
* Aneuploidy, including syndromes such as Down's, by the detection of an abnormal concentration of a particular chromosome.

Several methods were discussed in this review, including detection of paternally inherited SNP[1]s (discussed August 2008 by Dhallan et al), the RNA-SNP allelic ratio method (discussed August 2008 by Lo et al) and high-throughput shotgun sequencing (described below). However the authors concluded that only sex determination and RhD diagnosis are nearing translation into clinical practice for high-risk individuals. In the long-term, the authors felt that there was a place for an appropriate technology for the analysis of cffNA which may form part of a prenatal screening programme.

[1] SNP: single nucleotide polymorphism. DNA sequence variations that occur when a single nucleotide (A, T, C, or G) in the genome sequence is altered.

November 2009 - Safety and Effectiveness Issues

After the publication of the papers by Dhallan et al and Lo et al in 2008, Fan et al (2008) proposed that it should be possible to use *digital* polymerase chain reaction[2] (PCR) to create a test for foetal aneuploidy that was independent of polymorphisms. However, due to the low levels of foetal DNA (<10%) against the high maternal DNA background, this has to date proved technically challenging and unfeasible. As an alternative method, the Stanford group used direct shotgun sequencing, followed by mapping to the chromosome of origin to sequence cell free DNA from the plasma of pregnant women.

With shotgun sequencing large pieces of isolated target DNA (in this case DNA purified from maternal plasma) are sheared or physically broken into small fragments of varying size. Shearing is a random process and some of the fragments will overlap. Appropriate sized fragments (2,000 base-pairs or 2kb) are then isolated using gel purification. The 2kb fragments are then cloned into a vector and transformed into E coli for amplification of the clones, creating a sequencing library. The sequence of each clone in the library, of which there are 100s, is then determined by an automated DNA sequencer. Finally the sequenced fragments are assembled into one full length sequence using a computer algorithm which finds overlapping or continuous sequences of the fragments. Shotgun cloning will usually result in some gaps between contigs because some sequences are missing from the library by chance (Updegraff 2009). Follow this link for an animated overview of this technique.

Maternal plasma was obtained from a mixed population of 18 women, known to contain both normal and aneuploidy pregnancies (trisomy 13, 18 and 21) (level III-2 diagnostic evidence). The gestational age of the subjects at time of sampling ranged from 10 to 35 weeks. Blood samples were obtained immediately after amniocentesis or chorionic villus sampling. For each sample an average of 10 million 25-bp sequence tags were obtained and an average of 154,000, 135,000 and 65,700 sequence tags were mapped to chromosomes 13, 18 and 21 respectively. Shotgun sequencing successfully identified all nine women carrying a trisomy 21 foetus from the nine women who were disomy 21 (Figure 1). A 99 per cent confidence interval was constructed of the distribution of the sequence tag density of chromosome 21, with the dashed line in Figure 1 representing the upper boundary. Using the upper bound of the confidence interval as a threshold for detecting trisomy 21, the minimum fraction of foetal DNA that would be detected was approximately two per cent. The nine trisomy pregnancies outliers in the distribution were statistically significant compared to the

[2] Digital Polymerase Chain Reaction is a refinement of conventional PCR methods that can be used to directly quantify and clonally amplify nucleic acids. Digital PCR is useful in the detection and quantification of low-level pathogens, rare genetic sequences, gene expression in single cells, and the clonal amplification of nucleic acids for the identification and sequencing of mixed nucleic acids samples such as foetal DNA in a maternal serum sample. http://en.wikipedia.org/wiki/Main_Page

disomy pregnancies ($p < 10^{-5}$). Plasma from two women carrying a trisomy 18 foetus and from one woman carrying a trisomy 13 foetus was also analysed. Over-representation of chromosomes 18 and 13 were observed, although the construction of a representative distribution was not possible due to the small number of cases. Despite the small numbers, both the trisomy 18 and 13 were statistically significant outliers compared to the disomy samples, $p<10^{-7}$ and $p<0.05$ respectively.

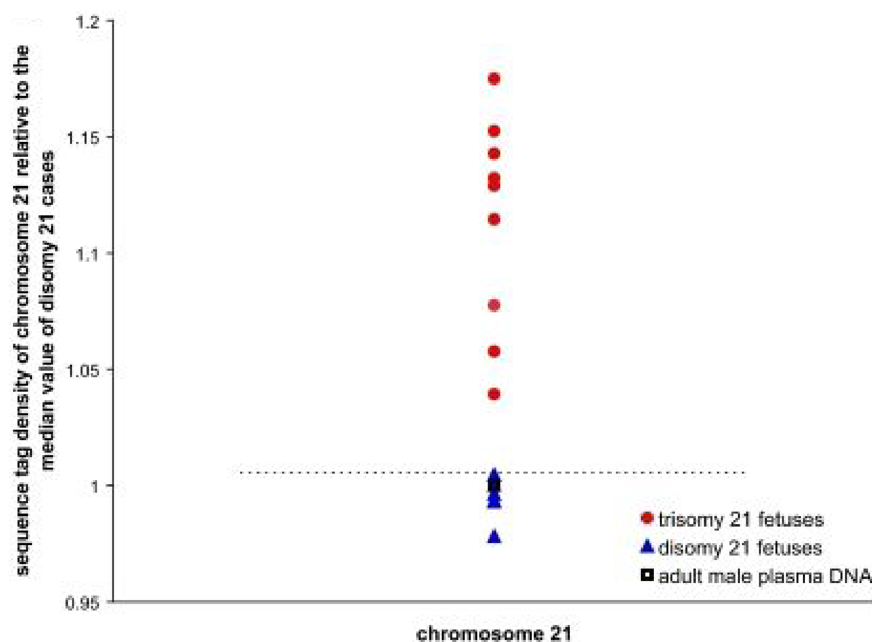

Figure 1 Chromosome 21 sequence tag density relative to the median chromosome 21 sequence tag density of normal cases (Fan et al 2008).

Note: In the paper discussed below, Chiu et al (2008) reviewed the paper by Fan et al (2008). The trisomy 21 samples in the Fan et al study were taken at 18 weeks gestational age, compared to the disomy samples which were taken much earlier at 12 weeks. In addition, all samples were taken within 15-30 minutes of amniocentesis or chorionic villus sampling. Foetal DNA increases significantly in the maternal circulation immediately after invasive procedures such as amniocentesis and also with pregnancy progression, which may confound the results reported by Fan.

A similar technique was employed by Chiu et al (2008), however this research group referred to their technique as parallel genomic sequencing. As in the Fan et al (2008) assay, target DNA was obtained from maternal plasma containing small amounts of foetal DNA (red fragments) (Figure 2). One end of each DNA fragment was sequenced for 36 bp and the chromosomal origin was identified via mapping to the human reference genome. The number of unique sequences mapped to each chromosome was counted and expressed as a percentage of all unique sequences and z-scores were calculated. It is expected that pregnancies with an aneuploid foetus will have a higher z-score (those indicated by the green bars) compared to the normal foetus (shown in the blue bar).

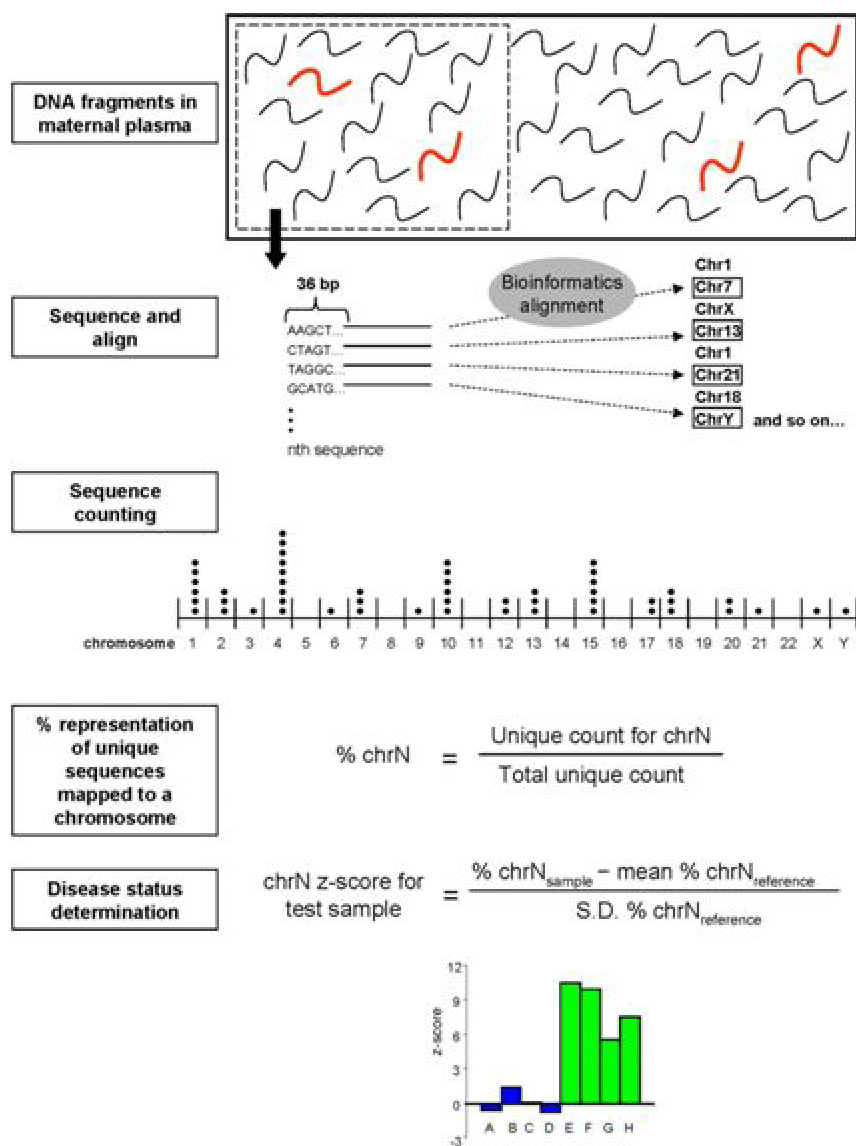

Figure 2 Schematic illustrating the use of parallel genomic sequencing (Chiu et al 2008)

Chiu et al obtained plasma samples from 14 women carrying a trisomy 21 foetus and 14 women pregnant with a normal foetus (mean gestational age 14.1 weeks). Chromosomal status was confirmed by full karyotyping (level III-2 diagnostic evidence). To objectively quantify the amount of over-representation in the chromosome 21 sequences of the trisomy foetuses, data from the normal male foetuses were used as a reference population. The z-scores for chromosome 21 and the X chromosome are summarised in Figure 3. For chromosome 21, all of the trisomy 21 foetuses had a z-score of >3 standard deviations above the normal reference sample (range 5.03-25.11). Therefore all trisomy 21 and disomy foetuses were correctly identified using this technique.

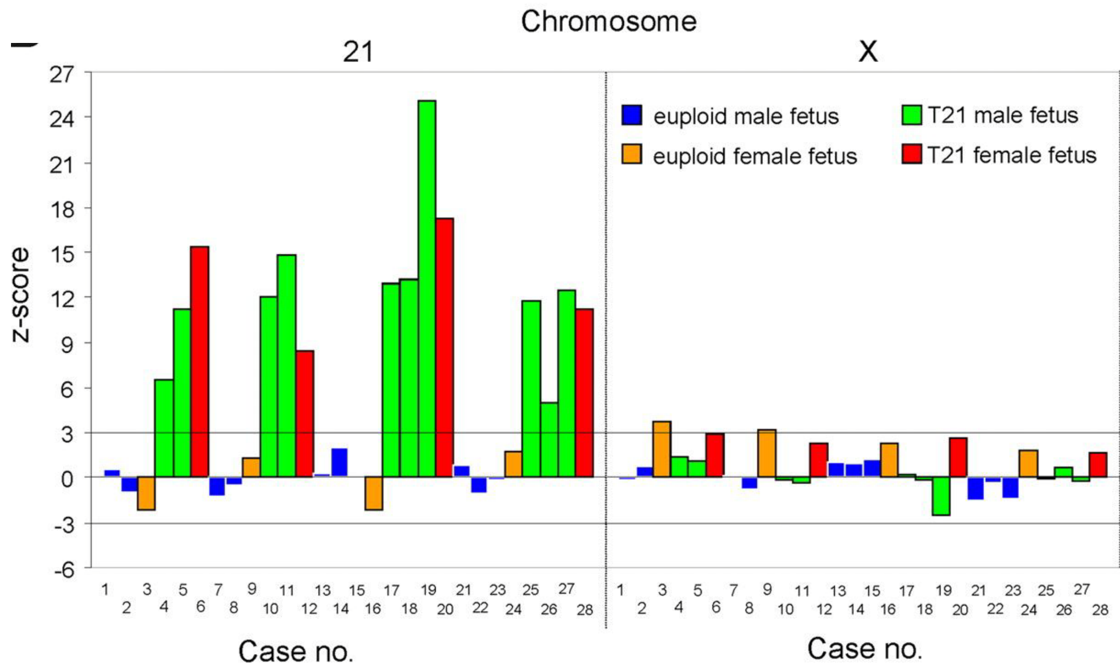

Figure 3 Plot of the z-scores for trisomy 21 foetuses (green and red) compared to normal foetuses (blue and yellow) for chromosome 21 and the X chromosome (Chui et al 2008)

NOVEMBER 2009 COST IMPACT

Fan et al (2008) estimate the cost of their shotgun sequencing assay to be approximately US$700, with the cost of sequencing expected to decrease in the future. These technologies are technically challenging, complex molecular techniques which would require a great deal of expertise and a suitable molecular pathology laboratory.

NOVEMBER 2009 ETHICS

NIPD only requires a small sample of blood and can be performed relatively easily, raising ethical, social and legal issues. As the test can be performed very early on in gestation without risk to the mother or foetus it may become a more common practice than amniocentesis. Obtaining a true informed consent may become difficult over time if these techniques become routine practice, which may lead to undue pressure on the mother once test results are known (Wright & Burton 2009). Counselling measures may need to be updated if these diagnostic advances become routine practice. The incidence of Down's Syndrome may increase with increasing maternal age at conception. Many women reject the offer of prenatal screening and routine offers of screening without careful counselling may hinder informed decision making (Mattheis et al 2008).

November 2009 Other issues

The review by Wright and Chitty (2009) lists a number of sites with further information on NIPD:

* Special Non-Invasive Advances in Fetal and Neonatal Evaluation Network (www.safenoe.org) - European network of research.
* Reliable Accurate Non-Invasive Prenatal Diagnosis (www.rapid.nhs.uk).
* PHG Foundation (www.phgfoundation.org) - Report of the UK working group.

November 2009 HealthPACT action:

Several techniques for the non-invasive prenatal diagnosis of foetal chromosomal aneuploidy appear to be feasible and have promise. Although there is an obvious and unmet need to perform non-invasive foetal tests at the earliest possible gestational time, these techniques are still in development. Whether or not the small-scale studies reviewed in the 2008 and 2009 prioritising summaries can be translated into routine clinical practice remains to be seen. Therefore HealthPACT have recommended that this summary be disseminated throughout the jurisdictions and that no further assessment is warranted at this time, as once the tests are suitable for routine clinical use they will be picked up by normal horizon scanning activity.

November 2009 Number of included Studies

Level III-2 diagnostic evidence	2

November 2009 References:

Chiu, R. W., Chan, K. C. et al (2008). 'Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma', *Proc Natl Acad Sci U S A,* 105 (51), 20458-20463.

Fan, H. C., Blumenfeld, Y. J. et al (2008). 'Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood', *Proc Natl Acad Sci U S A,* 105 (42), 16266-16271.

Mattheis, P. J., Hickey, F. et al (2008). 'Prenatal diagnosis: beyond decisions about termination', *J Pediatr,* 153 (5), 728; author reply 728-729.

Updegraff, E. (2009). *What is shotgun sequencing?* [Internet]. Conjecture Corporation. Available from: http://www.wisegeek.com/what-is-shotgun-sequencing.htm [Accessed 23rd September].

Wright, C. F. & Burton, H. (2009). 'The use of cell-free fetal nucleic acids in maternal blood for non-invasive prenatal diagnosis', *Hum Reprod Update,* 15 (1), 139-151.

Wright, C. F. & Chitty, L. S. (2009). 'Cell-free fetal DNA and RNA in maternal blood: implications for safer antenatal testing', *BMJ,* 339, b2451.

PRIORITISING SUMMARY (2008)

REGISTER ID: **000303**

NAME OF TECHNOLOGY: **NON-INVASIVE PRENATAL DIAGNOSTIC TEST FOR TRISOMY-21 (DOWN'S SYNDROME)**

PURPOSE AND TARGET GROUP: **FOR THE DETECTION OF FOETUSES WITH TRISOMY-21**

STAGE OF DEVELOPMENT (IN AUSTRALIA):

- ☒ Yet to emerge
- ☐ Experimental
- ☐ Investigational
- ☐ Nearly established
- ☐ Established
- ☐ Established *but* changed indication or modification of technique
- ☐ Should be taken out of use

AUSTRALIAN THERAPEUTIC GOODS ADMINISTRATION APPROVAL

- ☐ Yes
- ☐ No
- ☒ Not applicable

ARTG number

INTERNATIONAL UTILISATION:

COUNTRY	LEVEL OF USE		
	Trials Underway or Completed	Limited Use	Widely Diffused
Austria	✓		
Hong Kong	✓		
United States	✓		
Canada	✓		

2008 - IMPACT SUMMARY:

The use of free foetal DNA isolated from maternal plasma may be a non-invasive prenatal testing method to identify woman who may be carrying a foetus with trisomy-21, otherwise known as Down's Syndrome (DS).

2008 - BACKGROUND

The majority of pregnant women are offered prenatal screening for foetal abnormalities such as Down's Syndrome in the form of an ultrasound for the detection of Nuchal translucency (thickness at the back of the neck in the foetus associated with DS) and markers in maternal serum. The results of these tests aim to identify women may be at risk of carrying a DS foetus, however a definitive diagnosis can only be made by *invasive* procedures, such as amniocentesis or chorionic villus sampling (see

Comparators section), which both carry an inherent procedure-related risk to the foetus (Saller & Canick 2008). In addition, these techniques are associated with a five per cent false positive rate and a detection rate for true positives of 64-96 per cent (Benachi & Costa 2007).

Several techniques are currently in development to enable the non-invasive prenatal diagnosis of DS. The 2007 Lancet paper by Dhallan et al presented the preliminary results of a study which isolated free foetal DNA from maternal serum. Diagnosis of foetal abnormalities using free foetal DNA is problematical due to the small volumes of free foetal DNA found in the maternal serum (approximately 3.4%) and the difficultly of distinguishing foetal DNA from maternal DNA. The use of formaldehyde during sample processing may increase the free foetal DNA yield to 25 per cent or more.

To distinguish foetal DNA from maternal DNA, Dhallan et al proposed using *multiple* single nucleotide polymorphisms (SNPs)[3] or mutations. For example, in Figure 4 only one SNP is considered. The green strand represents the maternal DNA which is homozygous for guanine (G/G). The blue strand represents paternal DNA which is homozygous at the same site for a different nucleotide, in this case thymine (T/T). The foetus must inherit one copy of each chromosome from each parent, therefore the foetal genome at that particular SNP site will be heterozygous (G/T). Therefore the presence of a thymine (Figure 4C) at the SNP site indicates the presence of a foetal signal. When quantifying the allele[4] ratios for SNPs on chromosome 21, if there were twice as many SNPs in the

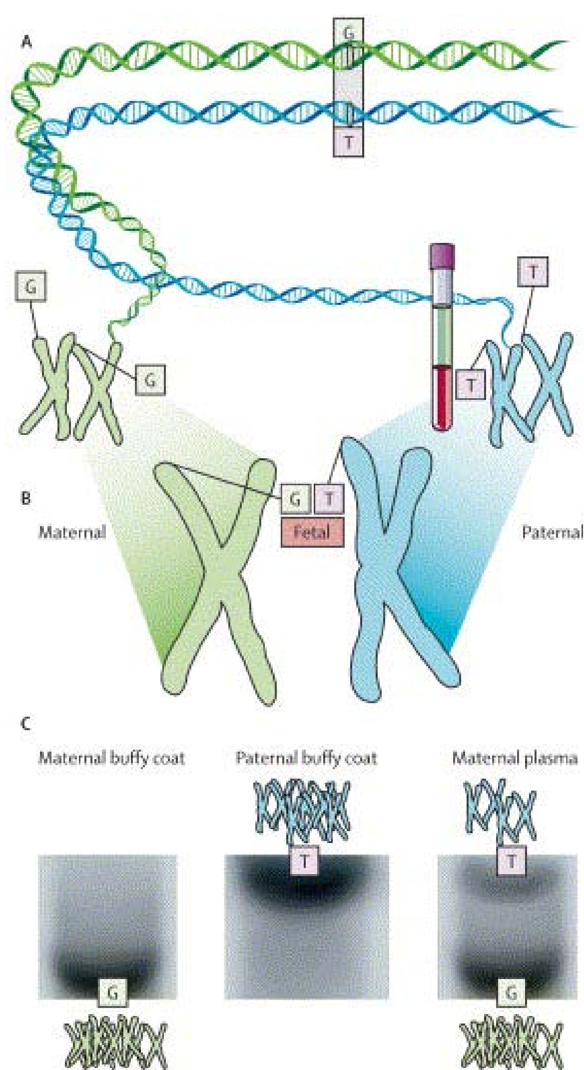

Figure 4 Inheritance of a single nucleotide polymorphism (Dhallan et al 2007)

[3] SNP: single nucleotide polymorphism. DNA sequence variations that occur when a single nucleotide (A, T, C, or G) in the genome sequence is altered.
[4] Allele: Alternative form of a gene. One of a different form of a gene that exists at a single locus.

foetal sample that match the paternal code than those matching the maternal code, then the disproportion would indicate that the foetus has an extra copy of chromosome 21 inherited from the father (Dhallan et al 2007).

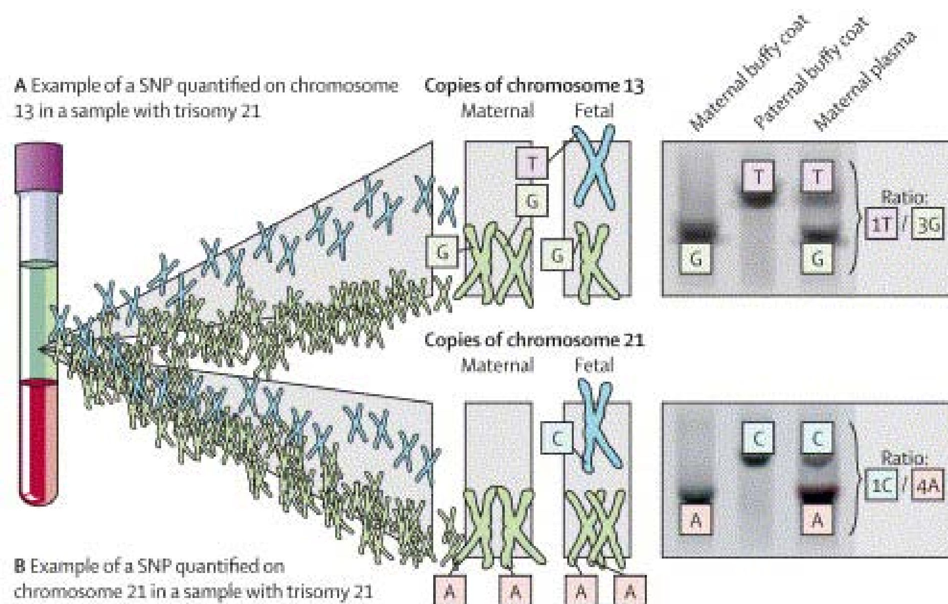

Figure 5 Analysis of allele ratios in maternal plasma (Dhallan et al 2007)

Figure 5 illustrates the quantification of SNPs on chromosome 13 in a sample with trisomy-21. In Figure 2A, the foetus inherits one maternal and one paternal copy of chromosome 13, therefore the ratio is 1T:3G. Over multiple SNP sites the mean ratio will approach 0.333. In Figure 2B with trisomy-21, the foetus inherits two maternal copies and one paternal copy of chromosome 21, and therefore the foetal allele ratio will approach 1:4 (0.25) over multiple SNPs (Dhallan et al 2007).

Another technique which shows great promise is based on the Nature paper by Lo et al (2007) and is in commercial development (SEQureDx™) by the company Sequenom (Lo et al 2007; Sequenom 2008). This technique is based on the isolation of messenger RNA (mRNA) of foetal origin released into the maternal circulation, following some of the basic principles as outlined by Dhallan et al. Gene expression patterns from pre-and post-natal maternal blood samples were examined. Those genes expressed in pre-natal but not post-natal samples were determined to be of foetal origin. The chromosome 21 encoded gene, placenta specific 4 (PLAC4), was highly expressed and could be detected in all three trimesters of pregnancy. Figure 6 describes the basic principle of the RNA-SNP allelic ratio method. When an SNP is transcribed the foetus with trisomy-21 will have an extra copy of the gene (Figure 6a). The gene is then expressed in the placental tissue and the ratio of the two RNA alleles in the trisomy-21 placenta will differ from the normal placenta (Figure 6b). When the transcripts are released into the maternal circulation, the difference in allelic ratios is reflected in the abundance of the transcripts, that is the level of circulating placental RNA will be greater in trisomy-21 samples (Figure 6c).

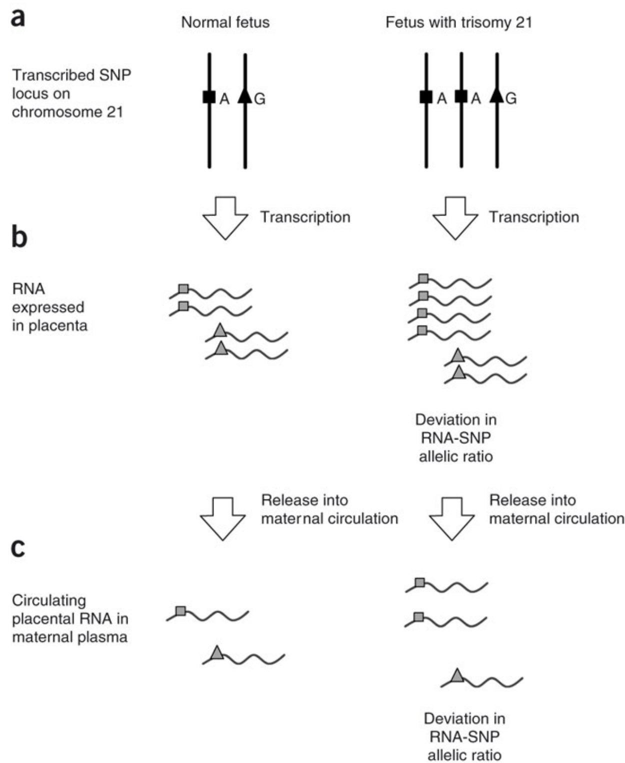

Figure 6 The RNA-SNP allelic ratio method (Lo et al 2007)

After RNA extraction from maternal blood, samples undergo RT-PCR followed by primer extension. Mass spectrometry is then conducted on these extension products.

2008 - Clinical Need and Burden of Disease

The last full report produced by the National Perinatal Statistics Unit (NPSU) contained data collected on birth anomalies during the period 1981-1997. In 1997, for the whole of Australia, there were 254,390 live births and 1,808 still births. During this same period there were a total of 4,489 congenital malformations (single and multiple), translating to a prevalence rate of 175 per 10,000 births[5]. The rate for chromosomal malformations was 22.5 per 10,000 births, with the majority of these being trisomy-21 or DS (13.0 per 10,000 births) (AIHW & UNSW 2001).

More recent data are available from birth defects registers from the individual states (Victoria, South Australia and Western Australia). In South Australia during the period from 1986-2003, there was a significant increase in the prevalence of DS, associated with increasing maternal age. In 2003, 12,603 pregnancies (72% of all pregnancies) were screened for DS. During this period there were 41 notified cases of DS, translating to a rate of 1.8 per 1,000 total births. Ten cases were detected in the first trimester by maternal serum screening (MSS) and Nuchal translucency (NT), 10

[5] Data includes congenital malformations diagnosed in liveborn infants in the first 28 days, or in still births of at least 20 weeks gestation or 400 gram birth weight. Terminations of pregnancies at≥ 20 weeks gestation are included

cases were detected in the second trimester by MSS, nine cases were detected by amniocentesis without prior screening and three cases by ultrasound. Seven cases were not detected by screening. Two of these cases were missed by NT and MSS and seven did not undergo any form of screening. Of the 41 cases, 31 pregnancies were terminated and 10 (24%) went on to a live birth (Haan et al 2004).

Similarly the Western Australian Birth Defects Registry reported in 2006 there were 74 cases of DS translating to a total rate (livebirths, stillbirths and terminations of pregnancy) of 2.6 per 1,000 pregnancies. The rate for liveborn DS infants is approximately 1 per 1,000 births (Bower et al 2006).

In Victoria the prevalence of DS has also been reported to be increasing primarily due to increasing maternal age. During the period 1989-1992 there were 16.6 cases of DS per 10,000 total pregnancies compared to 28.6 per 10,000 total pregnancies for the period 2001-04. In 2003-04, the prevalence rate per 10,000 pregnancies for DS increased steadily from 8.5 for women aged 25-29 years, to 15.9 for those aged 30-34 years and 53.3 for woman aged 35-39. Woman aged >40 years had a prevalence rate of 203 per 10,000 pregnancies. During 2003-04, there were 360 cases of DS detected by screening. Of these 65 per cent of DS pregnancies were terminated at less than 20 weeks gestation with seven per cent terminated at greater than 20 weeks gestation. Over time, there has been no decline detected in Victoria of the number of babies liveborn with DS, with 27 per cent of DS pregnancies during 2003-04 going on to a live birth (Riley & Halliday 2006).

In New Zealand during 2003, there were 56,134 registered live births (New Zealand Health Information Service 2007). New Zealand data indicates in 2003, that of a total of 4,657 malformations, 55 were Down's Syndrome (NZ Birth Defects Monitoring Programme, personal communication).

2008 - DIFFUSION

These techniques are not currently in use in Australia or New Zealand for the diagnosis of DS. However, many molecular pathology laboratories would have the expertise to perform these techniques (DNA isolation, PCR etc). In addition, there are approximately 10 mass spectroscopy units in Australia capable of performing nucleotide analysis with the Sequenom technology including the Victorian Clinical Genetics Services (VCGS) in the Murdoch Children's Research Institute. Sequenom estimate that the non-invasive diagnosis of DS will be commercially available in the United States by mid-2009 (personal communication Sequenom).

2008 - COMPARATORS

Two-dimensional ultrasonography is used as a routine screening tool for all pregnancies in Australia and New Zealand and may be used to diagnosis foetal abnormalities such as DS. A pre-12-week ultrasound is recommended in some cases including recurrent (>2) abortion or a previous foetal abnormality. Women considered

to be at risk of carrying a DS foetus (>35 years) may be offered an ultrasound between 11 and 13 weeks gestation for the detection of nuchal translucency (RANZCR 1998).

In addition, some foetal anomalies may be detected through analytes in the maternal serum at approximately 15-20 weeks of gestation. For example, the South Australian Maternal Serum Antenatal Screening Programme measures four analytes in maternal serum (α-fetoprotein, free alpha subunit (α-hCG) and free beta subunit (β-hCG) of chorionic gonadotropin, and unconjugated oestriol), which are normally produced by, or in support of, a foetus and its placenta. These analytes have known values throughout each stage of pregnancy. A number of foetal abnormalities result in one or more of these analytes deviating (increasing or decreasing) from their normal range. Seventy-four per cent of DS cases are detected using MSS. If these biochemical values fall outside of the normal range then the foetus is deemed to be at an *increased risk* of having an abnormality and mothers are offered counselling along with further information (SAMSAS 2005).

Pregnant women may be offered further invasive testing including amniocentesis and chorionic villus sampling (CVS), to confirm suspected foetal defects obtained either via an ultrasound scan or maternal biochemical testing. Some women may be offered these procedures based on maternal age alone. In South Australia maternal age was a factor in 57 and 63 per cent of all amniocenteses and CVSs, respectively, performed during 2003 (Haan et al 2004). Both of these techniques are deemed to be highly accurate but carry a small increased risk of miscarriage (0.5-2.6%). Amniocentesis can only be carried out at 15-17 weeks gestation, whereas CVS can be performed at 10-11 weeks. The time taken for cytogenic diagnosis can range from 1-3 weeks and this delay may have implications if the woman is considering termination. In addition, foetal blood sampling (cordocentesis or percutaneous umbilical blood sampling) may be performed from as early as 12 weeks, but usually after 16 weeks, gestation (Haan et al 2004).

2008 - SAFETY AND EFFECTIVENESS ISSUES

In a multi-centre study, Dhallan et al (2007) recruited 60 women (median age 34 years, range 18-43 years) with a singleton pregnancy (median gestation 17 weeks and 5 days, range 8 – 37 weeks). Approximately 35 ml (range 25-50 ml) of blood was taken and genomic DNA was isolated from both the mother and father of the baby. Amniocentesis or clinical assessment was used as the reference standard (level III-2 diagnostic evidence). Chromosome 13 was used as a reference standard as it is rarely associated with foetal abnormalities. SNPs were amplified from the isolated genomic DNA by polymerase chain reaction (PCR). The mean proportion of free foetal DNA isolated from the maternal plasma samples was 34.0 per cent (range 17.0-93.8%) The majority of the samples (51/60, 85%) had >25 per cent free foetal DNA. A mean of 22

(range 7-46) and 20 (range 8-43) SNPs were analysed on chromosome 13 and 21, respectively.

A significant difference in the ratio of foetal DNA to maternal DNA was observed in three samples (samples 4, 18 and 31) (Table 1). After amniocentesis or clinical postnatal clinical assessment, copy number was determined correctly in 58/60 (96.7%) of samples. The new diagnostic method correctly identified 56/57 (98%) of the normal samples and 2/3 (66.7%) of the trisomy-21 samples (samples 4 and 31). In sample four the mean ratio for foetal DNA for chromosome 21 was *significantly higher* that that for chromosome 13, indicating trisomy-21 and that the additional copy was inherited from the *paternal genome*. The mean ratio of foetal DNA for chromosome 21 was *significantly lower* in sample 31 compared to chromosome 13, indicating trisomy-21 in the foetus with the additional copy inherited from the *maternal genome*. The sensitivity of the new method was 66.7 per cent (95% CI [12.5, 98.2]) and the specificity was 98.2 per cent (95% CI [89.4, 99.9]). The false positive rate was 1.8 per cent, compared to the five per cent for conventional diagnostic methods. Sample 18 was a negative result by amniocentesis and was therefore considered to be a false positive. Amniocentesis identified sample 55 as trisomy-21, however the new method classified this as a normal sample and therefore a false negative.

Table 1 Comparison of mean foetal DNA ratios for chromosomes 13 and 21

Sample	Chromosome	Number of SNPs quantified	Ratio of foetal to maternal DNA	Difference in foetal DNA ratio (13 vs 21)	p value
4	13	46	0.8826	-0.3621	0.04
	21	35	1.2446		
18	13	11	0.1218	-0.0772	0.05
	21	10	0.1990		
31	13	34	0.2704	0.0877	0.04
	21	23	0.1827		
55	13	25	0.1887	-0.0310	0.34
	21	23	0.2197		

Initial analysis by Lo et al (2007) was conducted on 42 normal and 12 trisomy-21 *placental tissue* samples. All trisomy-21 samples had RNA-SNP allelic ratios which deviated from the normal samples and a reference interval was determined (mean RNA-SNP ratio for normal samples ± 1.96 standard deviations). Only one trisomy-21 sample fell inside this reference interval, translating to a sensitivity and specificity of 91.7 and 100 per cent, respectively. This method was then applied to 57 maternal blood samples from women known to be carrying a normal foetus (mean gestation 13 weeks, range 11.1-14 weeks) and 10 women carrying a trisomy-21 foetus[6] (mean gestation 14.7 weeks, range 12.4-20 weeks). Two of the normal samples fell *outside* of the normal reference interval and one of the trisomy-21 samples fell *inside* the

[6] Method of trisomy-21 determination not stated

normal reference interval, translating to a sensitivity and specificity of 90 and 96.5 per cent, respectively (level III-3 diagnostic evidence) (Lo et al 2007).

Several other authors have reported on non-invasive prenatal diagnostic techniques (Bauer et al 2006; Chim et al 2008; Go et al 2008; Krabchi et al 2006; Puszyk et al 2008).

2008 - COST IMPACT

To date there has been no costing information published on this technology. It has been estimated that these methods will be cheaper and more rapid than sequencing. The cost of a basic mass spectrometry unit capable of performing the techniques described in this summary is approximately AUD$350-375,000 (personal communication Sequenom).

2008 - ETHICAL, CULTURAL OR RELIGIOUS CONSIDERATIONS

There are obvious ethical concerns surrounding the use of prenatal testing, the results of which may result in the termination of a pregnancy. Woman need to be offered appropriate counselling and sufficient information as to the likelihood of a false positive or false negative result.

2008 - OTHER ISSUES

No issues were identified/raised in the sources examined.

2008 - SUMMARY OF FINDINGS

The isolation of foetal RNA and DNA from maternal blood appears to be feasible for the non-invasive diagnosis of Down’s Syndrome. Current diagnostic techniques have detection rates for true positives ranging from 64-96 per cent and false positive rates of five per cent. The DNA technique proposed by Dhallan et al resulted in a low false positive rate of only 1.8 per cent, however the true positive detection rate was also low at 67 per cent. Although the preliminary results reported by Lo et al using circulating foetal RNA resulted in an improved true positive detection rate of 90 per cent, the false positive rate also increased to 3.5 per cent. However, these results should be treated with caution as the number of women enrolled in each study was low.

2008 - HEALTHPACT ACTION:

Foetal DNA and RNA isolation from maternal blood samples appears to be a feasible technique for the non-invasive determination of trisomy-21. Both tests need to be optimised for their routine use in prenatal testing and large-scale studies should be conducted. Therefore HealthPACT recommended that this technology be monitored for further information in 12-months time.

NUMBER OF INCLUDED STUDIES

Total number of studies	
Level III-3 diagnostic evidence	1
Level III-2 diagnostic evidence	1

2008 - REFERENCES:

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SEARCH CRITERIA TO BE USED:

Chromosome Disorders/blood/*diagnosis/genetics
*Chromosomes, Human, Pair 21
DNA/*blood
Female
Humans
Maternal-Fetal Exchange
Polymorphism, Single Nucleotide
Pregnancy
Prenatal Diagnosis
Trisomy/*genetics