



Australian Government
Department of Health and Ageing



Australia and New Zealand Horizon Scanning Network

ANZHSN

AN INITIATIVE OF THE NATIONAL, STATE AND
TERRITORY GOVERNMENTS OF AUSTRALIA
AND THE GOVERNMENT OF NEW ZEALAND

Horizon Scanning Technology Prioritising Summary

Non-invasive prenatal testing for Down's Syndrome

August 2008



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ISBN

Publications Approval Number:

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The production of this Horizon scanning prioritising summary was overseen by the Health Policy Advisory Committee on Technology (HealthPACT), a sub-committee of the Medical Services Advisory Committee (MSAC). HealthPACT comprises representatives from departments in all states and territories, the Australia and New Zealand governments; and ASERNIP-S. The Australian Health Ministers' Advisory Council (AHMAC) supports HealthPACT through funding.

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PRIORITISING SUMMARY

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):

- | | |
|---|---|
| <input checked="" type="checkbox"/> Yet to emerge | <input type="checkbox"/> Established |
| <input type="checkbox"/> Experimental | <input type="checkbox"/> Established <i>but</i> changed indication or modification of technique |
| <input type="checkbox"/> Investigational | <input type="checkbox"/> Should be taken out of use |
| <input type="checkbox"/> Nearly established | |

AUSTRALIAN THERAPEUTIC GOODS ADMINISTRATION APPROVAL

- | | |
|--|-------------|
| <input type="checkbox"/> Yes | ARTG number |
| <input type="checkbox"/> No | |
| <input checked="" type="checkbox"/> Not applicable | |

INTERNATIONAL UTILISATION:

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

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).

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 difficulty 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)¹ or mutations. For example, in Figure 1 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 1C) at the SNP site indicates the presence of a foetal signal. When quantifying the allele² ratios for SNPs on chromosome 21, if there were twice as many SNPs in the

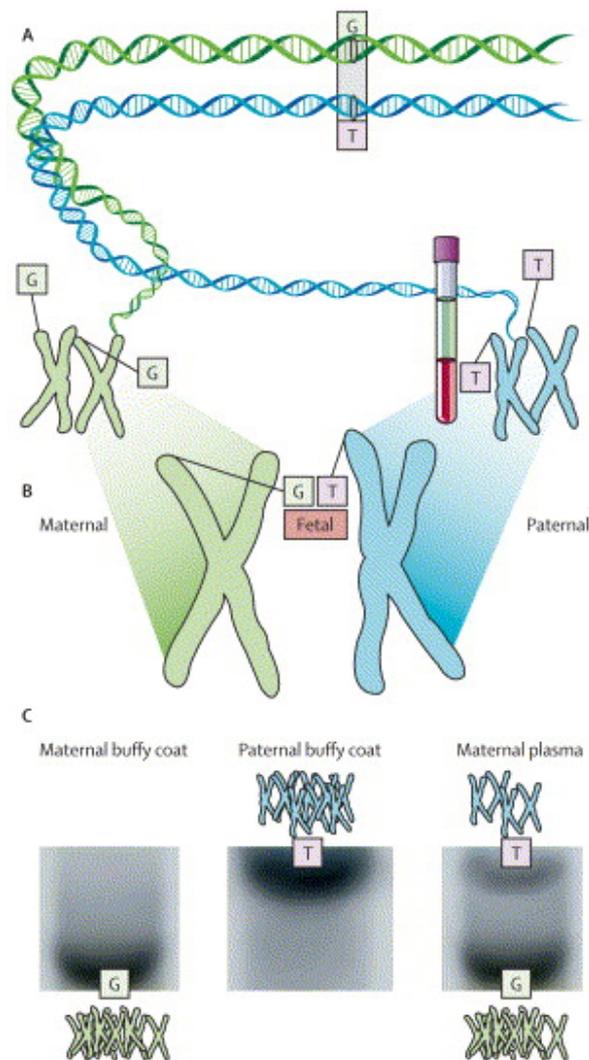


Figure 1 Inheritance of a single nucleotide polymorphism (Dhallan et al 2007)

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.

² 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).

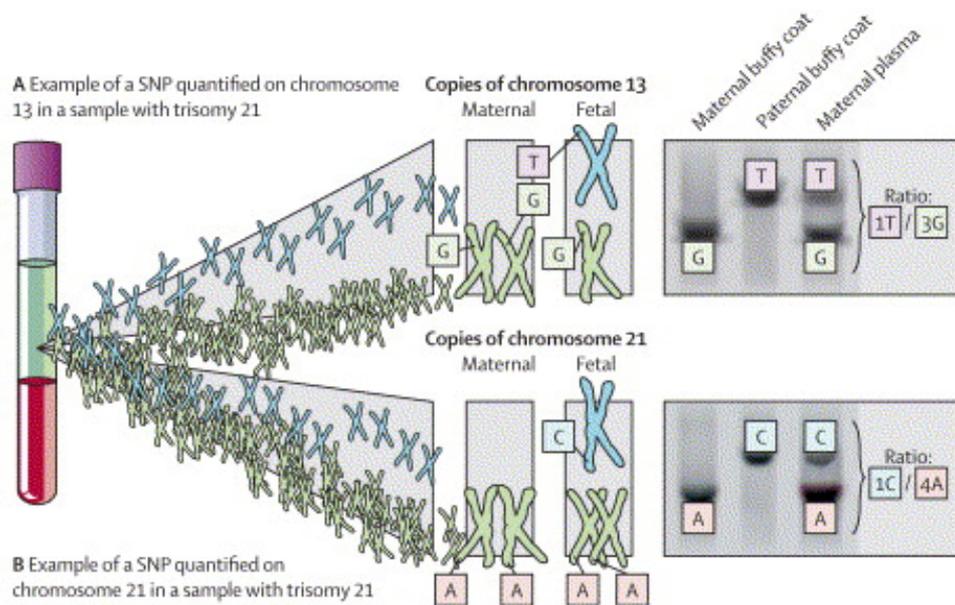


Figure 2 Analysis of allele ratios in maternal plasma (Dhallan et al 2007)

Figure 2 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 (SEQuereDx™) 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 3 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 3a). 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 3b). 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 3c).

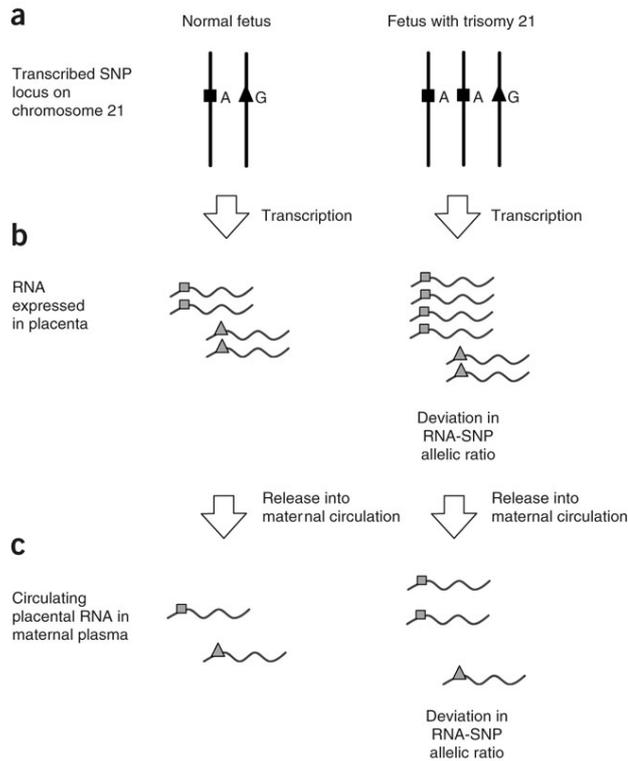


Figure 3 The RNA-SNP allelic ratio method (Lo et al 2007)

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

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 births³. 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

³ 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).

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).

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).

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* than 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 \pm 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 foetus⁴ (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

⁴ 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).

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).

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. Women need to be offered appropriate counselling and sufficient information as to the likelihood of a false positive or false negative result.

OTHER ISSUES

No issues were identified/raised in the sources examined.

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.

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

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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