

## **Combining first and second trimester markers for Down syndrome screening – think twice.**

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## **Abstract**

**Aims:** This study compares different screening strategies for the detection of Down syndrome and considers practical implications of using multiple screening protocols.

**Methods:** The performance characteristics of each screening strategy were assessed based on datasets of Down syndrome (n=11) and unaffected pregnancies (n=1,006) tested in both first and second trimester, as well as data from first trimester (n=18,901) and second trimester (n=40,748) pregnancies.

**Results:** For a detection rate of 91%, the false positive rates for integrated and serum integrated screening were 2.5% and 6.3% respectively, compared with combined first trimester (4.6%) and second trimester (12.6%) screening. Contingent and sequential screening protocols achieved detection rates of 82 to 91% with false positive rates between 2.6 and 2.9%. Contingent protocols require re-testing of 15 to 20% of cases in the second trimester. Sequential and integrated protocols require re-testing of 98 to 100% of cases in the second trimester. The various screening strategies did not always detect the same Down syndrome pregnancies.

**Conclusions:** Combining first and second trimester markers for Down syndrome screening better defines the at risk population. However integrated protocols complicate management of screening programs and may not be suitable as primary screening strategies. It may be a better use of resources to refine current first and second trimester programs through improved access and new markers. We therefore suggest thinking twice before embracing integrated population screening programs.

## Introduction

There have been population-based second trimester antenatal screening programs for Down syndrome and other fetal anomalies in Australia since 1991<sup>1</sup>. First trimester combined screening was introduced in 2000 in Australia and several studies<sup>3-6</sup> have demonstrated first trimester program performances (Detection Rate [DR] 83-87% for a 5% False Positive Rate [FPR]) comparable with international groups<sup>7-9</sup>. The majority (78%) of women who undertook screening tests in Australia in 2004 opted for first trimester screening<sup>2</sup>.

Multiple factors determine which programs women access. Availability of appropriate ultrasound facilities, gestational age at presentation, awareness that there is a choice of screening tests, doctor preference and hospital guidelines all play a part. Currently Australian policy guidelines recommend that women should be made aware of the availability of first and second trimester screening tests for Down syndrome and other chromosomal abnormalities<sup>10</sup>. However, professional organisations in Canada<sup>11</sup>, the United States<sup>12</sup> and the United Kingdom<sup>13</sup>, have proposed that women be given a choice of alternative strategies that include integrated<sup>14</sup>, contingent<sup>15 16</sup> or sequential<sup>17</sup> screening.

The Down syndrome screening protocols reviewed are outlined in Table 1.

Integrated, contingent and sequential protocols are based on fetal nuchal translucency thickness (NT) and maternal serum PAPP-A measurements in first trimester and either triple or quadruple maternal serum markers in the second trimester.

*Integrated screening* involves withholding risk odds until second trimester testing is complete<sup>8</sup>. This approach is controversial since women do not have the option of early diagnosis following increased risk first trimester results, leading some to question whether this can be considered ethical practice<sup>18</sup>.

*Contingent screening* involves issuing two risks<sup>15 16 19</sup>. Women are first stratified according to both a high risk ( $\geq 1/50$ ) and low risk cut off ( $< 1/1500$ ) following a first trimester screen. High risk women are offered CVS or amniocentesis, low risk women are not offered further testing. Those of intermediate risk ( $1/51 - 1/1500$ ) are offered second trimester testing after which an integrated risk is issued.

*Sequential screening* a variation of contingent screening involves offering invasive diagnostic testing to women screened in first trimester to be at increased risk ( $>1/30$  or  $> 1/63$ ); the remaining women are offered second trimester screening for an integrated risk assessment<sup>17 20</sup>.

Contingent and sequential screening by definition will have slightly higher FPR than integrated screening but all three will have lower FPR than both first and second trimester screening. All three integrated protocols require re-testing in the second trimester and will therefore incur increased running costs<sup>21</sup>.

Cautionary policy advice from the United Kingdom NHS<sup>22</sup> and USA<sup>19 23 24</sup>, has highlighted that integrated protocols pose too many practical problems to be introduced at present.

The question is whether Australia can support a range of screening options and what might be the policy implications for societal uptake, altered clinical and program management and cost effectiveness. In this paper we compare different screening strategies for the detection of Down syndrome and consider practical implications of using multiple screening protocols.

## **Methods**

A prospective and retrospective review of pregnancies screened by the South Australian Maternal Serum Antenatal Screening (SAMSAS) Program was performed to compare various Down syndrome screening strategies. These data include 11 pregnancies with fetal Trisomy 21, identified from screened pregnancies with matched first and second trimester serum samples and marker results. For these pregnancies, only first trimester combined Down syndrome risk odds expressed at term had been issued. These cases were compared with 1,006 unaffected pregnancies with matched first and second trimester serum samples and marker results that were used to establish integrated distributions and assess FPR. These 1,006 unaffected cases had been issued a first trimester combined Down syndrome risk and a second trimester risk for neural tube defects but not a second trimester Down syndrome risk. Maternal serum samples from 64 unaffected pregnancies screened at increased risk by the first trimester combined screen, which had a matched second trimester serum sample and marker results, were used as controls to assess the impact of integrated distributions on the FPR. A further 18,901 first trimester combined screened pregnancies and 40,748 second trimester triple test-screened pregnancies were also used for comparative data. Ascertainment of cases and program performance have been reported previously<sup>5 25</sup>.

Data were compiled as part of comprehensive annual audits maintained with approval from the Women's and Children's Hospital Research Ethics Committee. Separate ethics approval was obtained to allow the collection and analysis of matched first and second trimester samples.

Stepwise discriminant analyses were performed using the statistical software package SPSS v 15.0 (2006 SPSS Inc.). Individual likelihood ratios were generated from overlapping Gaussian distributions and risk odds were calculated using the conventional algorithm<sup>1 26 27</sup> incorporating the maternal age risk at term adjusted for gestation in days<sup>28 29</sup>. Risk odds generated have been empirically validated<sup>30 31</sup>. A Down syndrome risk odd of  $\geq 1/300$  at term was used for both the first trimester combined and the second trimester triple test as the cut off risk for diagnostic testing.

First trimester free  $\beta$ -hCG measurements were not used in integrated distributions as stepwise discriminatory analysis showed that their contribution was not significant. By omitting the first trimester free  $\beta$ -hCG, Wald et al (2006) estimated less than 0.1% decrease in FPR at detection rates up to 90%<sup>21</sup>.

The contribution to the rate of invasive prenatal diagnostic testing made by screening programs was estimated as a screening-diagnostic index (SDI). The SDI is derived from the percentage of women screened at increased risk (4.9% first trimester, 7.2% second trimester) multiplied by the uptake of diagnostic tests following an at increased risk report (~80% for both) multiplied by the screening uptake for each screen expressed as a percent of total confinements (49% first trimester, 25% second trimester in 2005)<sup>2 5</sup>.

Performance characteristics for the various screening strategies were calculated for different risk odds.

## Results

Coefficients and distributions for integrated and serum integrated screens have been newly derived from the cohorts described in this paper. Performance of the different screening tests, by applying these distributions to the 11 affected and 1,006 unaffected cases, with matched first and second trimester serum samples and markers is summarised in Tables 1, 3, 4 and 5.

From 64 unaffected cases screened at increased risk by the first trimester combined screen, we were able to demonstrate that integrated and serum integrated tests reduced the FPR by 44% (36 remained at increased risk) and 39% (39 remained at increased risk), 000respectively. The same magnitude of reduction was observed in the cohort of 1,006 cases (Tables 4 and 5).

Table 3 shows that different screening strategies did not always detect the same Down syndrome affected pregnancies. Cases 8, 10 and 11, would be given discrepant risk odds depending on the gestation at which they presented for screening and the screening test used. Case 11 would not be offered further testing according to contingent screening protocols since the first trimester risk odds are less than 1/2000 and therefore this case is counted as a false negative.

Tables 2 and 4 show screening performance and cohort data from 18,901 first trimester and 40,748 second trimester screened pregnancies on which a single Down syndrome risk was issued using a risk cut off of  $\geq 1/300$  at term for both protocols. These data are used to compare cohorts and performance of screening strategies.

Direct comparisons between screening strategies, based on the cohort of 1,006 unaffected pregnancies, show a lower FPR for integrated (2.5%) versus first trimester combined (4.6%) screening and serum integrated (6.3%) versus the second trimester triple test (12.6%) (Tables 4 and 5). The higher FPR seen with the second trimester triple test for the cohort of 1,006 unaffected pregnancies is not unexpected as it is an older group (median age 31.2 years) than the prospective second trimester cohort of 40,748 pregnancies (median age 29.5 years). This observation highlights the important role of NT measurements in the risk calculation in keeping the FPR low and DR high<sup>32</sup>. Further comparison of serum integrated with prospective second trimester triple screening, demonstrates higher DR (90.9% versus 74.6%) for a similar FPR (6.3% versus 7.0%, Table 4).

For contingent protocols, 15.8% of cases had first trimester combined risk odds between 1/51 and 1/1500 and in 20.4% of cases had risk odds between 1/31 and 1/2000, using the prospective first trimester cohort of 18,901 pregnancies. Sequential protocols identified between 1.0% and 1.6% of cases with risk odds  $\geq 1/30$  and risk odds  $\geq 1/63$  respectively. Contingent and sequential protocols would require re-testing of around 15% and 98% of cases for second trimester markers, respectively.

Using contingent and sequential screening protocols we observed FPRs between 2.6% and 2.9% for DR 81.8 to 90.9% respectively, (Tables 1 and 3).

## Discussion

We have demonstrated by retrospective analysis that combining first and second trimester markers can result in a 91% DR for Down syndrome affected pregnancies for a substantially lower FPR (2.5%) than either first trimester combined (4.6% FPR and 90.9% DR) or second trimester triple tests (7.0% FPR and 74.6% DR ), consistent with other published data<sup>3 4 6 9 38</sup>.

Contingent screening protocols did not improve detection over first trimester combined (81.8% at risk cut off  $\geq 1/300$ ) and as shown in Table 3, different affected cases were detected. Cases 8, 10 and 11 generate discrepant risk odds and may or may not have been detected depending on the gestation at which they presented and the screening test used. These data demonstrate that if more than one screening strategy is in place and two risk odds are generated, then an “at increased risk” result cannot be ignored and a diagnostic test should be offered to these patients. Serial screening or the re-issuing of an independent second trimester risk odd following a first trimester combined screen should not be encouraged. The number of discrepant risk odds and the FPR would increase (the FPR being additive would approach or exceed 10%) and as it is difficult to ignore risk estimates once divulged, the percentage of diagnostic tests offered would be unacceptably high<sup>19</sup>. Contingent protocols result in 15-20% of cases being issued with two risk odds, increasing the chance of generating discrepant risk odds.

The dilemma with using integrated protocols is that they rely on variable risk cut offs (Table 1). Table 5 shows that a DR of 90.9% for a FPR of 2.5% can be achieved using integrated

distributions; however by simply adjusting the cut off risk one could lower the FPR to  $\leq 0.8\%$  and still achieve a DR of 72.7%. In practice, different risk cut-offs could be confusing, would require re-education of patients and health professionals and would complicate patient and program management.

The disadvantage of contingent and sequential protocols is that a first trimester risk needs to be calculated, but not necessarily reported in respect of nearly all patients. Whether these protocols are practical or even desirable is an open question<sup>21</sup>. Certainly it would be difficult to withhold a patient's first trimester risk odd if requested. Amongst pregnancies with odds between 1/301 and 1/2000 in contingent screening protocols, 0.8% of cases went from low risk to high risk (cut-off risk  $\geq 1/300$ ) when analysed using the integrated test, further adding to the pool of high risk results which patients and health professionals need to reconcile.

The effect of issuing two risks needs proper evaluation. In a program that focuses on minimising FPR, DR would decrease (72.7% Table 3), while one that focuses on maximising DR could potentially result in detections approaching 100% but with unacceptably high FPRs (Tables 3, 4 and 5).

SAMSAS offers both first and second trimester screening and issues one Down syndrome risk per pregnancy. Diagnostic testing is advised based on a risk cut off of  $\geq 1/300$  at term. With this strategy in place there has been a significant decrease in the proportion of confinements undergoing invasive diagnostic testing, from 9.3% to 7.6% between 1995 and 2005, with an accelerated decrease coinciding with the introduction of first trimester

combined screening in November 2000<sup>5</sup>. In 2005 the contribution of both first and second trimester screening to the invasive diagnostic rate was 3.3% as estimated by the SDI. The difference (4.3%) between the SDI and the rate of invasive diagnostic testing reflects additional factors such as women's attitudes, counselling, maternal age, previous history and ultrasound anomalies. It is also an indirect indication that women want reassurance if there is any suggestion that there may be any problem with their baby. The trend or choice appears to favour diagnostic testing<sup>41-43</sup>. Variable risk cut offs and intermediate risk groups as described in contingent or sequential protocols could inadvertently channel more women towards invasive testing, undoing the gains already achieved with current first and second trimester strategies that use single risk cut offs.

Integrated screening protocols still require obstetric scans and may reveal anomalies. Management of these pregnancies is best done in conjunction with a first trimester combined Down syndrome risk assessment<sup>8 39 40</sup>. Papers describing better performance characteristics of integrated screening overlook the importance of the first trimester Down syndrome risk and have not assessed the impact of non-disclosure. With-holding such information avoids problems with issuing two risk odds but the delay creates practical issues with compliance and delayed diagnosis. It also leads to risk assessments being made through sub-optimal combinations of markers that result in lower performance than predicted from statistical modelling. An interventional study on integrated screening published by Weisz<sup>38</sup> supports these statements. Weisz reports a DR of 100% (11/11) for a FPR of 4.6%. Only 59.3% (2027/3417) of the cohort actually had a full integrated screen and only after reminder letters were sent to 25% of the 3417 cohort that failed to present for the second sample. The other patients (40.7%) were issued risks based on six different

screening tests (NT  $\geq$  3.5mm, serum integrated, quadruple + NT, combined first trimester, NT + PAPP-A and quadruple).

Running costs of contingent strategies would be higher than first trimester combined screening by at least 15.8 – 20.4%, as this percentage of patients would be retested in second trimester. Sequential and integrated screening running costs increase by significantly more, as 98 – 100% of patients presenting in the first trimester would be retested in the second trimester.

Our findings are consistent with the view that integrated algorithms better define the at risk pregnant population. However, screening performance determined from modelling (which assumes 100% compliance) or from controlled clinical trials (with strict adherence to protocols) is unlikely to be matched by comparable performance in community-based programs, which are subject to many variables<sup>19</sup>.

We have raised issues against integrated protocols such as increased operational costs, poor compliance, variable risk cut offs, discrepant risk odds, increased maternal anxiety, increased counselling, late intervention and optimistically low FPR in a clinical setting. These considerations suggest that it may not yet be suitable as the primary population screening strategy.

We therefore suggest thinking twice before embracing integrated screening as an alternative population screening program. A better use of resources might be to refine current first and second trimester programs through improved access and information, new

markers (ADAM12, PP-13, absence of nasal bone, tricuspid regurgitation and abnormal ductus venous flow) and expanding these screening programs to include other significant pregnancy pathologies such as pre-eclampsia and fetal growth restriction<sup>8 39 40 44-53</sup>.

A limitation of this study is that the 11 cases of Down syndrome do not represent a uniform cohort. They are identified affected cases where both first and second trimester markers were available. Intervention normally occurs in pregnancies screened at increased risk, so the 11 cases may be biased towards first trimester false negatives. The strength of this study is that we have been able to assess the same pregnancy with known outcome using different screening protocols.

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Table 1. Down syndrome screening protocols defined including performance.

Screen <sup>#</sup>	1st Trimester Markers	Diagnostic Test Offered In 1 <sup>st</sup> Trimester	2 <sup>nd</sup> Trimester Markers	Risk Odds Reported	SAMSAS Performance <sup>@</sup> %DR (%FPR) Cut off Risk 1/300 at Term
First Trimester Combined	NT, PAPP-A, $\beta$ -hCG	Yes	n/a <sup>~</sup>	1 <sup>st</sup> Trimester	90.9 (4.6)
Second Trimester	n/a <sup>~</sup>	n/a <sup>~</sup>	AFP, total or free $\beta$ -hCG, uE3 (Triple) + Inhibin-A (Quadruple)	2 <sup>nd</sup> Trimester	74.6 (7.0)
Integrated	NT, PAPP-A	No	Triple or Quadruple Markers*	2 <sup>nd</sup> Trimester	90.9 (2.5)
Serum Integrated	PAPP-A	No	Triple or Quadruple Markers*	2 <sup>nd</sup> Trimester	90.9 (6.3)
Contingent	NT, PAPP-A, $\beta$ -hCG	Yes, if DS risk odds $\geq$ cut off risk (1/30 or 1/50) <sup>\$</sup>	n/a <sup>~</sup>	1 <sup>st</sup> Trimester	63.6 (0.7,1.2)
		No, if intermediate DS risk odds, (1/31 – 1/2000 or 1/51 -1/1500) <sup>\$</sup>	Triple or Quadruple Markers*	1 <sup>st</sup> and 2 <sup>nd</sup> Trimester	18.2 (2.0,1.4)
		No, if low risk, DS risk odds $<$ cut off risk (1/2000 or 1/1500) <sup>\$</sup>	n/a <sup>~</sup>	1 <sup>st</sup> Trimester	n/a <sup>~</sup>
Sequential	NT, PAPP-A, $\beta$ -hCG	Yes, if DS risk odds $>$ cut off risk (1/30 or 1/63) <sup>\$</sup>	n/a <sup>~</sup>	1 <sup>st</sup> Trimester	63.6 (0.7,1.3)
		No, if DS risk odds $<$ cut off risk (1/30 or 1/63) <sup>\$</sup>	Triple or Quadruple Markers*	2 <sup>nd</sup> Trimester	27.3 (2.0,1.6)

Abbreviations: NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein A;  $\beta$ -hCG, free beta subunit human chorionic gonadotropin; AFP, alpha-fetoprotein; uE3, unconjugated estriol; DS, Down syndrome; DR, detection rate; FPR, false positive rate

\*Triple serum screen incorporating AFP, total or free  $\beta$ -hCG and unconjugated estriol; quadruple serum screen also incorporates inhibin –A

<sup>#</sup>All screens include maternal age at term in the risk odds calculations.

<sup>~</sup> n/a, not applicable.

<sup>\$</sup>Various cut off risks have been described.

<sup>@</sup>Where second trimester markers are used, performance is based on the Triple serum screen with free  $\beta$ -hCG and does not include Inhibin-A.

Table 2. Down syndrome Detection Rates and False Positive Rates seen per Risk Odds category.

Risk Odd (1:n)	First Trimester Combined Screen		Second Trimester Triple Screen	
	Detection Rate % (no.)	False Positive Rate % (no.)	Detection Rate % (no.)	False Positive Rate % (no.)
≥ 50	74.2 (49/66)	1.1 (211/18835)	38.8 (26/67)	1.5 (611/40681)
≥ 100	83.3 (55/66)	1.8 (344/18835)	52.2 (35/67)	2.7 (1090/40681)
≥ 200	87.9 (58/66)	3.2 (609/18835)	65.7 (44/67)	4.9 (1974/40681)
≥ 300	90.9 (60/66)	4.6 (868/18835)	74.6 (50/67)	7.0 (2864/40681)
≥ 1500	97.0 (64/66)	17 (3205/18835)	94.0 (63/67)	25.0 (10156/40681)
≥ 3500	100% (66/66)	33.1 (6255/18835)	94.0 (63/67)	43.1 (17531/40681)
Median Maternal Age (years)	36.9	31.3	35.7	29.5
Median Gestation (weeks+days)	12w0d	12w2d	16w0d	16w1d

Table 3. Risk odds at term on 11 confirmed cases with Down syndrome (DS) where both first and second trimester markers were available.

T21 Case	Age at Delivery years	First Trimester #DS odds	Second Trimester #DS odds	Serum Integrated #DS odds	Integrated #DS odds
1	42.4	2	2	2	2
2	44.5	2	2	2	2
3	32.6	2	51	98	2
4	36.5	2	9	3	2
5	36.3	2	10	22	2
6	38.0	2	23	2	2
7	40.6	11	2	2	2
8	36.6	131	943	6136	974
9	25.3	193	140	26	160
10	32.5	396	44	36	9
*11	37.5	<b>*2076</b>	<b>*21</b>	<b>*104</b>	<b>*278</b>
Case(s) DS odds < 1/300		10,11	8	8	8
DR resulting if !minimising FPR			72.7%	72.7%	72.7%
DR resulting if !!maximising detection			100%	100%	100%
			with an ↑FPR (>10%) ↑Costs	with an ↑FPR (>10%) ↑Costs	with an ↑FPR (>7%) ↑Costs

Abbreviations: T21, Trisomy 21; DR, detection rate; DS, Down syndrome; FPR, false positive rate

# DS, Down syndrome odds at term

\* Case 11, first trimester DS risk is < 1/1500, under some protocols this patient would not be offered integrated screening and is counted as a miss for contingent screening.

! If discrepant risk odds are reported management focus is to accept a low risk result over a high risk result.

!! If discrepant risk odds are reported management focus is to accept a high risk result over a low risk result.

Table 4. Cohort comparative data and screening performance for Down syndrome detection\*.

	Prospective	Prospective	Prospective	Retrospective	Retrospective	Retrospective
	First Trimester combined	Second Trimester Triple	First Trimester combined	Second Trimester Triple	Serum Integrated	Integrated
Cohort	18,901 sequential	40,748 sequential	1,006 First with a Second trimester sample	1,006 Second with a First trimester sample	1,006 First and Second samples	1,006 First and Second samples
% Recall Rate	4.9	7.2	Unaffected	Unaffected	Unaffected	Unaffected
% FPR	4.6	7.0	4.6	12.6	6.3	2.5
% DR (no.)	90.9 (60/66)	74.6 (50/67)	81.8 (9/11)	90.9 (10/11)	90.9 (10/11)	90.9 (10/11)
% of Cohort with NT $\geq$ 2.5mm	3.0	-	2.4	-	-	-
% of Cohort with NT $\geq$ 3.0mm	1.3	-	0.9	-	-	-
% of Cohort with NT $\geq$ 3.5mm	0.7	-	0.6	-	-	-
% Cohort with DS odds between 1/31 and 1/2000	20.4	29.6	18.9	43.8	15.9	10.8
% Cohort with DS odds between 1/51 and 1/1500	15.8	23.5	14.6	35.3	13.3	8.9
Median Gestation (weeks+days)	12w2d	16w1d	12w2d	17w0d		
Median Maternal Age (years)	31.3	29.5	31.2	31.2		

Abbreviations: FPR, false positive rate; DR, detection rate; NT, nuchal translucency; DS, Down syndrome

\* Screening performance is based on a risk cut off of 1/300 at term

- Not applicable

Table 5. Screening performance of screening strategies on cases with matched first and second trimester samples per Risk Odds category.

Risk Odd (1:n)	First Trimester Combined		Second Trimester Triple		Serum Integrated		Integrated	
	DR %	FPR %	DR %	FPR %	DR %	FPR %	DR %	FPR %
≥ 50	63.6	1.2	72.7	1.9	72.7	1.9	72.7	0.8
≥ 100	63.6	1.8	81.8	4.6	81.8	2.8	72.7	1.1
≥ 200	81.8	2.9	90.9	8.8	90.9	4.4	81.8	2.0
≥ 300	81.8	4.6	90.9	12.6	90.9	6.3	90.9	2.5
≥ 1500	90.9	15.8	100	37.2	90.9	15.2	90.9	9.7
≥ 3500	90.9	29.2	100	60.4	90.9	24.1	90.9	16.3

Affected cases = 11, Unaffected cases = 1006.