Information for Health Professionals

about

Maternal Serum Screening

Information from the South Australian Maternal Serum Antenatal Screening Programmeã , Department of Chemical Pathology,
Women's and Children's Hospital, North Adelaide 5006.

Maternal Serum Screening and how it works

The biochemistry of a developing baby has considerable impact on that of its mother. Not surprisingly, when fetal development is abnormal, maternal biochemistry sometimes reflects this. The high maternal serum alphafetoprotein (AFP) concentration associated with an open fetal neural tube defect is probably the most familiar instance of this.

In screening for fetal disorders the South Australian Maternal Serum Antenatal Screening (SAMSASã) Programme measures four analytes in a mother's blood when she is between 15 and 20 weeks pregnant. These are alpha-fetoprotein (AFP), free alpha subunit (α-hCG) and free beta subunit of chorionic gonadotropin (β-hCG), and unconjugated estriol (uE3). These four analytes are normally produced by, or in support of, a fetus and its placenta and have known values at each stage of pregnancy.

A number of fetal abnormalities and pregnancy pathologies result in one or more of these analytes changing from their normal values. The normal pattern of results becomes disturbed. Examples of some of the abnormal patterns found are given later in this booklet. Maternal serum screening for fetal abnormalities thus comes down to comparing the biochemical results found in a given mother's blood with these
patterns. If the results match one of the patterns, then the biochemical results are taken to indicate that the fetus is at *increased risk* of having that particular abnormality. If the results for all analytes fall within normal limits, then it is concluded that the fetus is *not at increased risk* of having an abnormality.

Increased risk' and 'not at increased risk' results

If maternal serum screening results fell clearly outside normal values when fetal development was abnormal, then biochemical screening would be able to declare each pregnancy normal or abnormal with certainty. In practice this does not occur. Results found when fetal development is abnormal overlap with those found in normal pregnancy. Because of this overlap, maternal serum screening cannot give a certain answer to the question "is this pregnancy normal?". It can only declare the pregnancy more likely to be (*increased risk*) or less likely to be (*not at increased risk*) that of an affected fetus.

The measure of the SAMSAS Programme's uncertainty in declaring a pregnancy normal or abnormal is given by the risk odds quoted on its results reports. A report stating *increased risk of Down syndrome (1:50)* means that on average one out of every 50 such reports issued by the SAMSAS Programme will truly refer to an affected pregnancy. The other 49 pregnancies will be unaffected by fetal Down syndrome. Conversely, a report stating *not at increased risk (1:2600)*, means one pregnancy out of every 2600 receiving such a report will be affected by fetal Down syndrome. These figures reflect the extent to which the screening results from affected pregnancies overlap with those from the normal population in this condition.

In counselling, these risk odds are sometimes referred to as the 'woman's risk'. Note, however, the quoted figures are more properly regarded as a measure of the uncertainty in the assessment of the risk, and do not stem from anything the woman has or has not done.

The SAMSAS© Programme

The aim of the SAMSAS Programme is to provide accurate forewarning of certain fetal abnormalities and pregnancy pathologies to obstetricians, general practitioners and midwives as they manage the pregnancies under their care. To this end the SAMSAS Programme maintains a close liaison with ultrasonographers, health centres, antenatal clinics and counselling support structures.

Ordering maternal serum screening

Maternal serum screening is best ordered from the SAMSAS Programme using one of the Programme's request forms (call (08) 8204 7285 for a supply). These come in pads of 50 with each request form accompanied by a copy of the booklet ***"Information for Parents about Maternal Serum Screening".*** This booklet is provided to facilitate pre-test counselling about the procedure. It is for parents to take away with them.

(In the absence of a SAMSAS request form a standard pathology request form can be used. The instruction "send to WCH" should be clearly written upon the request form to ensure the request is forwarded to the SAMSAS Programme.)

Testing

Testing is performed every working day. Mothers can have their blood specimen taken at any one of the blood collection centres around the State. They do not have to come to the Women's and Children's Hospital for blood collection. Specimen required is 5-10mL clotted blood.

Increased risk results

When a pregnancy is found to be *at increased risk* of any of the screened conditions, a further information booklet is sent with the results report. **Experience has shown that giving families these booklets at the time they receive their report eases the way in which they deal with the information**.

- If the pregnancy is screened at increased risk of Down syndrome the booklet ***"Increased risk of Down syndrome - what does it mean?"*** is provided.
- If the pregnancy is screened at increased risk of an open neural tube defect the booklet ***"Increased risk of NTD - what does it mean?"*** accompanies the report.
- If an increased maternal serum AFP is found the booklet ***"Increased AFP - what does it mean?"*** is sent.

Further interpretation of results is available by telephone
((08) 8204 7285).

Reports and follow-up

Specimens are processed within one working day of receipt. Results are generally available by 4.00 pm by telephone.

All reports indicating *increased risk* of a screened condition are faxed to the referring doctor or clinic, with a written report following by post or courier.

Test Results
in Maternal Serum Screening

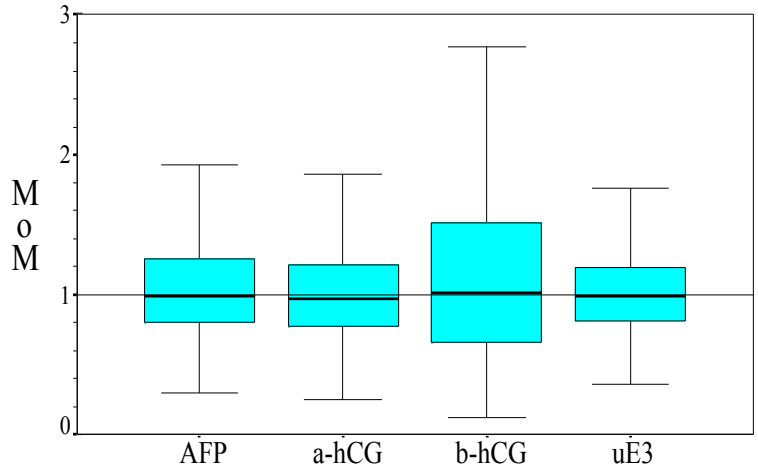
On the following pages are some of the patterns in test results recognised by the SAMSAS Programme.

The range of values found for the four analytes, alpha-fetoprotein (AFP), free alpha subunit (a-hCG) and free beta subunit (b-hCG) of chorionic gonadotropin, and unconjugated estriol (uE3), are shown in box plot form.

Because the serum concentration of each of these analytes changes as pregnancy progresses, values are expressed as Multiples of the population Median (MoM values) expected at the gestational age of the specimen being analysed. This allows the different values measured at different gestational ages to be compared directly with one another and interpreted consistently. For example, an AFP value of 41 KIU/L in the blood of a mother of 15 weeks gestation (where the median value for the population is 27 KIU/L) would be interpreted similarly to a maternal serum AFP value of 80 KIU/L in another mother who was of 20 weeks gestation (where the median value for the population is 53 KIU/L). Both results are 1.5 Multiples of the Median value expected for their gestational age, or 1.5 MoM.

Note that the normal value for any analyte at any gestational age within the screening window of 15-20 weeks is 1.0 MoM.

Screened Condition - **Normal Pregnancy**



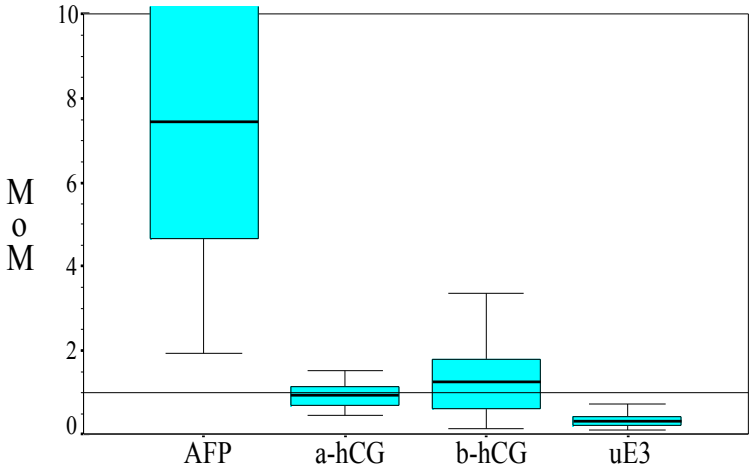
Population prevalence

96% of all accurately dated pregnancies give this pattern

Notes:

1. All analyte values vary about 1 MoM (Multiples of the population Median value for the gestational age of the pregnancy).
2. This is the pattern of analytes with which the results from each pregnancy are compared in screening for fetal abnormalities and pregnancy pathologies.

Screened Condition - **Anencephaly**

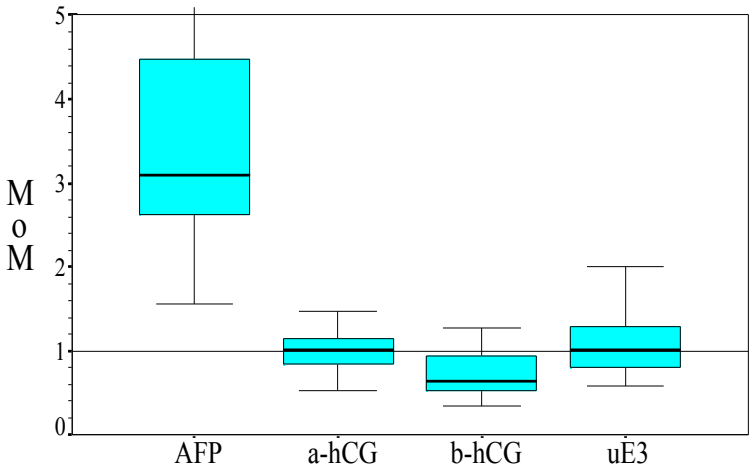


Population prevalence	1:1000
Detection by screening	93%
Screening recall rate	0.3%
Odds affected pregnancy given an "increased risk" result	1:3
Odds affected pregnancy given a "not at increased risk" result	<1:9999

Notes:

1. Characteristic pattern of high AFP and low uE3.
2. The SAMSAS Programme issues an "Increased risk of NTD" report.
3. Detailed ultrasound examination usually confirms the diagnosis.

Screened Condition - **Meningomyelocoele**

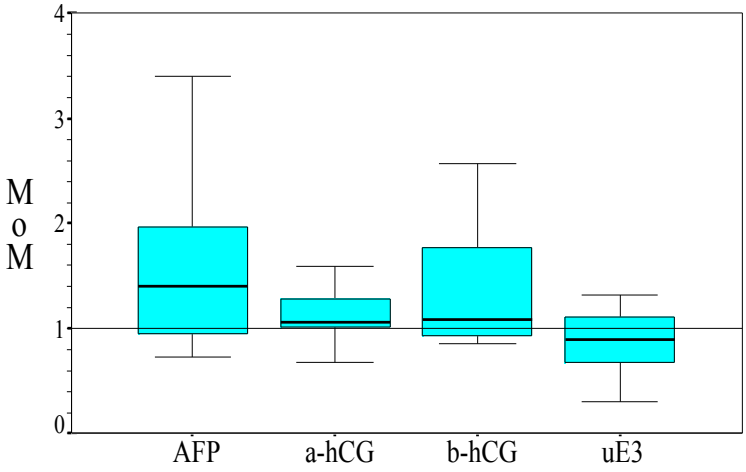


Population prevalence	1:1000
Detection by screening	92%
Screening recall rate	0.6%
Odds affected pregnancy given an "increased risk" result	1:7
Odds affected pregnancy given a "not at increased risk" result	<1:9999

Notes:

1. Characteristic pattern of raised AFP and lowered b-hCG result (not known why).
2. The SAMSAS Programme issues an "Increased risk of NTD" report
3. Diagnosis usually confirmed by detailed ultrasound examination. If necessary, AFP quantitation and secretory acetylcholinesterase detection in the amniotic fluid can be performed.

Screened Condition - **Encephalocoele**

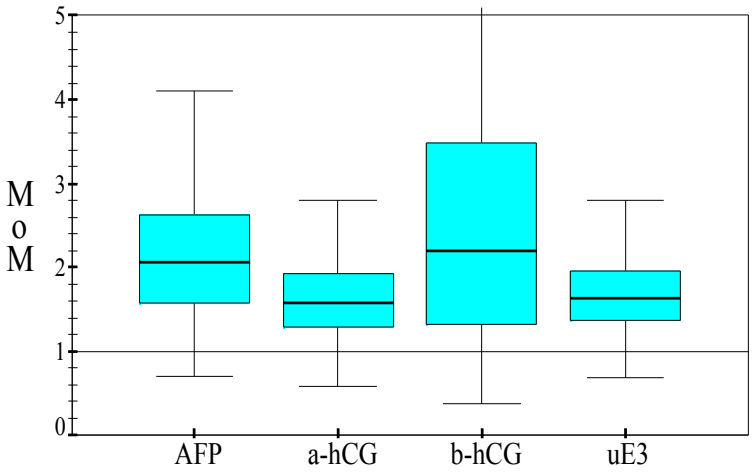


Prevalence in screened population 1:8000
Detection by screening 35%

Notes:

1. Poorly discriminated from normal pregnancies by biochemical screening.
2. Approximately 35% of affected pregnancies are associated with a raised maternal serum AFP value. For these, the SAMSAS Programme issues an "Increased AFP" report.
3. Detailed ultrasound examination offers the best chance of detection of all closed NTDs.

Screened Condition - **Twin Pregnancy**

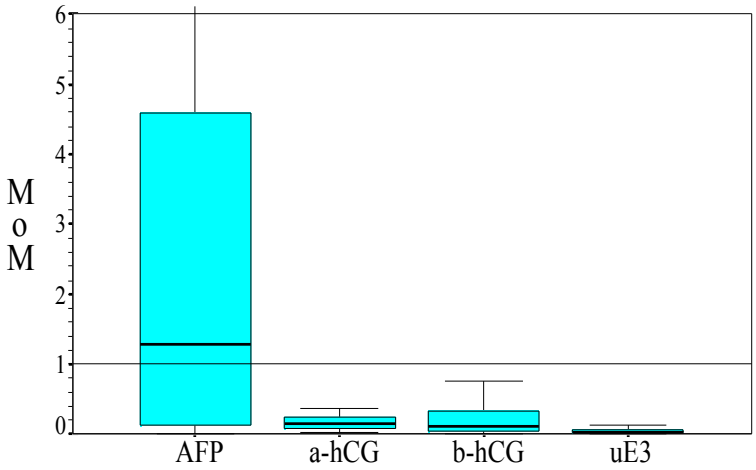


Population prevalence 1:80

Notes:

1. Not a formally screened condition but an occasional incidental finding.
2. Unsuspected multiple pregnancies are a frequent cause (1:30) of an elevated AFP result.
3. The raised values of all the other analytes helps distinguish multiple pregnancy from other conditions associated with raised AFP results.
4. The SAMSAS Programme issues an “Increased AFP” report.
5. Readily confirmed by ultrasound.

Screened Condition - **Failed Pregnancy**

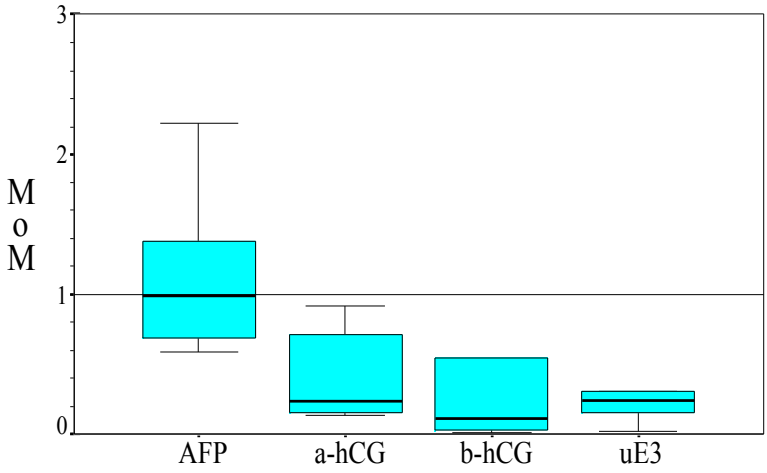


Prevalence in screened population	1:1000
Detection by screening	99%

Notes:

1. Not a formally screened condition but an occasional incidental finding.
2. Usually (but not always) indicated by a high AFP value but very low values for the other three analytes.
3. The SAMSAS Programme requests confirmation of the presence of a fetal heartbeat before issuing a report.

Screened Condition - **69XXX (Triploidy)**

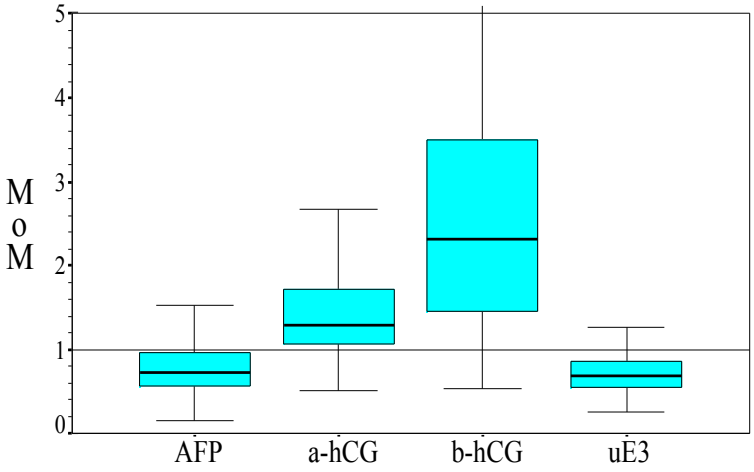


Prevalence in screened population 1:15000
Detection by screening 78%

Notes:

1. Pattern easily confused with that of a failed pregnancy (see previous page) except that there is a fetal heartbeat.
2. Not formally screened by the SAMSAS Programme. Results currently used in support of ultrasonography findings.

Screened Condition - **Trisomy 21 (Down syndrome)**

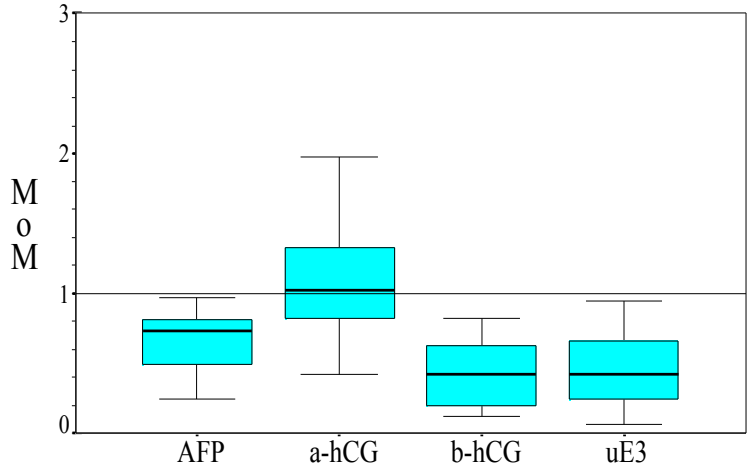


Prevalence in screened population 1:730
Detection by screening 74%
Screening recall rate 5.9%
Odds affected pregnancy given an "increased risk" result 1:58
Odds affected pregnancy given a "not at increased risk" result 1:2600

Notes:

1. Typically a distinctive pattern of low AFP and uE3 values coupled with raised a-hCG and b-hCG concentrations.
2. Detection rate and recall rate both vary with maternal age, being higher at more advanced ages.
3. This pattern is very similar to that observed in a pregnancy whose gestational age is overstated by two or more weeks (see over page).

Screened Condition - **Trisomy 18 (Edwards syndrome)**

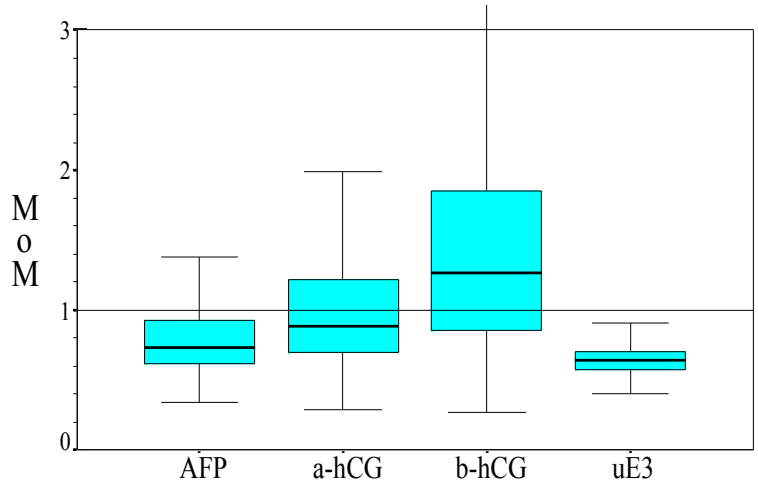


Prevalence in screened population	1:8500
Detection by screening	70%
Screening recall rate	0.4%
Odds affected pregnancy given an "increased risk" result	1:50
Odds affected pregnancy given a "not at increased risk" result	<1:9999

Notes:

1. A very distinct pattern of low values for three of the four analytes measured.
2. Not formally screened by the SAMSAS Programme at the present time.
3. Results currently used in support of equivocal ultrasonography findings.

Screened Condition - **Overstated Gestational Age**



Prevalence in screened population	1:100
Detection by screening	99.9% (if > 3weeks overstated)

Notes:

1. Not a formally screened condition.
2. The SAMSAS Programme issues a report requesting confirmation of gestational age if the biochemical results indicate a difference of 3 weeks or more from the stated gestation.
3. This pattern is very similar to that found when there is fetal Down Syndrome (see previous page) and is a major reason for false positive results in screening for this condition.

The Importance of Correct Gestational Age Assessment

Overstatement of gestational age is the greatest avoidable cause of false positive results in maternal serum screening for Down syndrome. Approximately 1% of specimens submitted to the SAMSAS Programme are from pregnancies overstated in gestational age by more than 3 weeks. **90% of these produce a result indicating *increased risk of Down syndrome.***

Gestational age assessments made from ultrasonographic measurements are the most reliable. Almost 80% of all corrections of gestational age called through to the SAMSAS Programme come from reports issued on pregnancies where the initial estimation is by dates or other unspecified method.

Any revision of the estimate of gestation requires a telephone call to the SAMSAS Programme ((08) 8204 7285) so that risks can be recalculated. This is important **whether or not the original report indicated increased risk of any condition.** Recalculation of risks is performed over the telephone. An amended report follows.

Correct assessments of gestational age mean more accurate assessments of risk from the SAMSAS Programme, fewer falsely *increased risk* reports for doctors and clinics to deal with, and less unnecessary anxiety for families.

Interpretation of Gestational Age Information by the SAMSAS© *Programme*

Gestational age information is presented to us in a number of ways. It is important that you give us your estimate of gestational age (in weeks and days) **and** the date upon which you made that estimate. The SAMSAS computer system will do the extrapolation to sample date. Transcription and calculation errors are then minimised.

For risk assessment we have adopted the following conventions when interpreting gestational age information on request forms:

if g.a. is stated as	***we read it as***
16 weeks on 21/2/98	16 weeks 0 days on 21/2/98
16+ weeks on 21/2/98	16 weeks 0 days on 21/2/98
16- weeks on 21/2/98	16 weeks 0 days on 21/2/98
15-16 weeks on 21/2/98	15 weeks 3 days on 18/2/98
15-17 weeks on 21/2/98	16 weeks 0 days on 21/2/98
15_ weeks on 21/2/98	15 weeks 3 days on 18/2/98
15-15_ weeks on 21/2/98	15 weeks 2 days on 19/2/98
15_-16 weeks on 21/2/98	15 weeks 5 days on 16/2/98
16^3 weeks on 21/2/98	16 weeks 3 days on 18/2/98
16.5 weeks on 21/2/98	16 weeks 3 days on 18/2/98
16.7 weeks on 21/2/98	16 weeks 5 days on 16/2/98

Given a choice of gestational age assessments, we have found the following hierarchy of methods to be most reliable

1 ultrasound;
2 certain LMP dating;
3 clinical estimation;
4 uncertain LMP dating.

The Use of Ultrasound Dating and 'increased risk' Results

The reason why ultrasound dating of pregnancy is given first place in the hierarchy of estimates shown on the previous page is given in the following table. It is found that the obstetric centres in South Australia which most frequently use

ultrasound to date pregnancies before requesting maternal serum screening also have the fewest corrections of gestational age information following the issuing of *'increased risk'* reports. Accurate initial dating produces fewer false positive results without reducing the detection of truly affected pregnancies (1996 SAMSAS Programme performance data).

Centre	% g.a. by u/s	% 'increased risk' results corrected for g.a. error
1	54.7	41.1
2	56.8	40.0
3	77.0	24.6
4	95.3	7.3
5	95.6	10.2

Performance of the SAMSAS© Programme

The SAMSAS Programme recognises that screening for abnormalities in pregnancy can generate considerable anxiety in parents and health professionals alike. Regrettably, false positive (and to a lesser extent false negative) results are unavoidable in screening. For this reason the performance of the SAMSAS Programme is continuously monitored. Annual audits of the SAMSAS Programme, now covering 85565 pregnancies screened since 1991, show that for the calender years 1994-7:

Year	Pregnancies screened	Recalled *'increased risk'* trisomy 21	Trisomy 21 detected
1994	11535	466 (4.0%)	11/15 (**73.3%**)
1995	11381	646 (5.7%)	11/17 (**64.7%**)
1996	12125	845 (7.0%)	12/15 (**80.0%**)
1997	11410	773 (6.8%)	22/27 (**81.5%**)

For Neural Tube Defects	
Pregnancies screened for NTD (1991-1997)	85 565
Recalled *'increased risk'* of NTD	2567 (**3.0%**)
NTDs Detected	89/103 (**86.4%**)

Uptake of Amniocentesis following an "increased risk" Report

Recommending amniocentesis to mothers aged 35 years or more at delivery would currently see this service offered to 13.5% of the pregnant population of South Australia (SA Pregnancy Outcome Report 1996). Characteristically about 51% of these would act upon the offer. In addition about 3% of mothers less than 35 at delivery would also request amniocentesis.

With an *'increased risk'* result from maternal serum screening, the uptake of amniocentesis in these two groups in the past three years has been as follows:

	Pregnancies screened	Declared *'increased risk'*	Proceeded to amniocentesis
1995			
< 35 years	10553	453 (4.3%)	342 (75.5%)
≥35 years	828	193 (23.3%)	128 (66.3%)
1996			
< 35 years	11188	594 (5.3%)	489 (82.3%)
≥35 years	937	251 (26.8%)	173 (68.9%)
1997			
< 35 years	10445	563 (5.4%)	483 (85.8%)
≥35 years	965	210 (21.8%)	175 (83.3%)

Maternal age and risk

In screening for fetal Down syndrome, maternal age is well recognised as part of the risk assessment. The following table shows the likelihood that a pregnancy is affected from the risk report issued by the SAMSAS Programme according to the mother's age at delivery (1997 Programme performance data).

Maternal age years	Maternal age risk 1:xxxx	Predicted detection rate %	Predicted recall rate %	Risk odds affected increased risk 1:xx	not increased risk 1:xxxx
18	1661	57.9	3.6	103	3795
19	1618	58.3	3.7	102	3738
20	1577	58.7	3.8	101	3675
21	1538	59.1	3.8	100	3616
22	1499	59.5	3.9	99	3556
23	1460	59.9	4.0	99	3495
24	1425	60.6	4.3	99	3462
25	1389	61.0	4.4	99	3406
26	1353	61.4	4.5	98	3349
27	1319	61.8	4.6	97	3296
28	1285	62.2	4.7	96	3241
29	1253	62.6	4.8	95	3191
30	1221	63.3	5.0	95	3162
31	978	66.6	6.3	93	2743
32	785	70.5	7.9	88	2450
33	630	73.6	9.7	83	2155
34	505	76.7	12.1	80	1905
35	405	80.0	14.5	73	1732
36	325	82.9	17.9	70	1560
37	260	85.8	21.5	65	1438
38	210	87.9	25.5	61	1293
39	165	90.2	30.2	55	1176
40	135	91.9	34.5	51	1092
41	105	93.6	40.1	45	982

The data quoted in this booklet are those from the South Australian Maternal Serum Antenatal Screening (SAMSASã) Programme, operating in the Department of Chemical Pathology of the Women's and Children's Hospital, Adelaide, South Australia. They do not apply to other maternal serum testing centres. Medical practitioners and other health professionals are advised to request the maternal serum screening performance figures from the centre performing the testing on their patients should that testing not be done by the SAMSASã Programme.