



Antenatal Screening

Antenatal Screening for Down's Syndrome
and Open Neural Tube Defects

The Integrated Test

Information for Health Professionals

The Wolfson Institute of Preventive Medicine
Barts and The London School of Medicine and Dentistry

Antenatal Screening

SUMMARY

- The purpose of screening is to identify women with an increased risk of having a pregnancy with Down's syndrome or an open neural tube defect so that they can be offered a diagnostic test.
- The integrated test is the most effective method of screening for Down's syndrome. The test is performed in two stages. In the first stage two measurements are made, the concentration of serum Pregnancy Associated Plasma Protein-A (**PAPP-A**) and the nuchal translucency (**NT**) using an ultrasound scan examination. In the second stage four serum levels are measured; alpha-fetoprotein (**AFP**), unconjugated oestriol (**uE₃**), free beta human chorionic gonadotrophin (**free β -hCG**) and **inhibin-A**.
- The measurements of these six markers are used together with the woman's age to estimate the risk of having a pregnancy with Down's syndrome. Women with a risk of 1 in 150 or greater are interpreted as screen-positive for Down's syndrome and offered a diagnostic test, usually an amniocentesis. About 1 in 65 of all women screened fall into the screen-positive group, and about 1 in 6 women with screen-positive results have an affected pregnancy.
- The level of AFP alone is used to screen for open neural tube defects. Women with a raised AFP level (equal to or greater than two and a half times the normal median) are interpreted as screen-positive and offered further tests such as an ultrasound scan. About 1 in 100 of all women screened fall into the screen-positive group for an open neural tube defect, and about 1 in 16 women with screen-positive results have an affected pregnancy.
- The integrated test detects 9 out of 10 cases of Down's syndrome and AFP measurement identifies 4 out of 5 cases of open spina bifida and nearly all cases of anencephaly. About 1 to 2% of women screened are offered a diagnostic test.
- Measurements used as part of the integrated test can also identify pregnancies at high risk of Edwards' syndrome (trisomy 18). The test identifies about 6 out of 10 cases of Edwards' syndrome.



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MAIN ADVANTAGES OF THE INTEGRATED TEST

- Safest and most effective method of screening for Down's syndrome
- Low false positive rate with high detection rate
- Integrates all the relevant information into a single test, avoiding the confusion associated with multiple screening
- Less anxiety. With most other screening tests about 1 in 20 women have a positive screening result. With the integrated test this is reduced to about 1 in 65.
- Includes AFP screening for open neural tube defects
- Permits identification of pregnancies at increased risk of Edwards' syndrome (trisomy 18)

DOWN'S SYNDROME (TRISOMY 21)

Down's syndrome is the most common cause of severe learning disability in children. It arises from an extra copy of chromosome 21 in the cells of the fetus. In the absence of antenatal screening, about 1 in 500 babies born would be affected.

People with Down's syndrome have varying degrees of learning disability, but usually the disability is severe. Some people will lead semi-independent lives while others will

be completely dependent. About 40% of Down's syndrome pregnancies will miscarry between 11 weeks and term, but nine out of ten affected babies who reach term will survive their first year. About 40% of babies with Down's syndrome are born with a serious heart defect. The average life expectancy of a person with Down's syndrome is now about 60 years, although most will develop pathological changes in the brain associated with Alzheimer's disease after the age of 40.

OPEN NEURAL TUBE DEFECTS

Neural tube defects are one of the most common serious congenital malformations. In the absence of antenatal screening, about 1 in every 650 babies born would be affected, or less than 1 in 1200 among women who took folic acid supplements immediately before becoming pregnant.

Anencephaly, one type of open neural tube defect, is fatal at, or within hours of birth, but the natural history of spina bifida is variable. Babies born with open spina bifida, the other main type of open neural tube defect, are often severely handicapped and can require several surgical procedures and hospitalisation. Disability typically consists of weakness or paralysis of the legs, urinary and faecal incontinence, hydrocephaly, and, less often, learning disability.



EDWARDS' SYNDROME (TRISOMY 18)

Edwards' syndrome is a rare (birth prevalence about 1 in 7000) and usually fatal abnormality which arises from an extra copy of chromosome number 18 in the cells of the fetus.

TIMING OF THE INTEGRATED TEST

Stage 1

The first stage of the test is best performed in the first trimester at 11 weeks of pregnancy, but any time between 10 and 13 completed weeks is acceptable, as determined by the first day of the woman's last menstrual period (LMP) or an ultrasound scan, if one has already been performed.

An ultrasound scan examination is carried out to determine the gestational age of the pregnancy by performing a fetal crown rump length measurement and to perform a nuchal translucency (NT) measurement. At about the same time a sample of blood is taken to measure the level of pregnancy associated plasma protein-A (PAPP-A), and a recommended date is given for the second blood sample.

Stage 2

The second stage of the test is best performed in the second trimester at 15 or 16 completed weeks of pregnancy but up to 22 weeks is

possible. A second sample of blood is taken to measure the level of alpha-fetoprotein (AFP), unconjugated oestriol (uE_3), free beta human chorionic gonadotrophin (free β -hCG) and inhibin-A.

An integrated test result can only be given if a woman provides blood at both stages. If a second blood sample is not received by the end of the 16th week a reminder will be sent to the woman's home address or to her General Practitioner (GP) or antenatal clinic. If the second sample is not received by the end of the 20th week the risk of Down's syndrome will be estimated using information from the first stage of the test only. This test is less effective than the integrated test.

BLOOD SAMPLE REQUIREMENTS

For the first stage of the test 10ml of clotted blood is required and is taken at the same time as the ultrasound scan and sent to the laboratory. For the second stage of the test a 10ml blood sample should be taken by the GP or antenatal clinic staff and sent to the laboratory.

INTERPRETATION OF THE INTEGRATED TEST

The test categorises women into two groups: screen-positive with a high risk of having an affected pregnancy and screen-negative with a lower risk of having an affected pregnancy.

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Screen-positive

i) Screen-positive for Down's syndrome

A woman is screen-positive if her risk of having a pregnancy with Down's syndrome based on the maternal age, the levels of PAPP-A, free β -hCG, AFP, uE₃, inhibin-A and the NT measurement, is estimated to be 1 in 150 or greater. About 1 in 65 screened women will be in this group.

ii) Screen-positive for open neural tube defects

If the AFP level is equal to, or greater than, two and a half times the normal median level (2.5 MoM), the result is screen-positive. About 1 in 100 women will be in this group.

Screen-negative

A screen-negative result means that (i) the risk of a pregnancy with Down's syndrome is below the specified cut-off or (ii) the AFP level is less than 2.5 MoM. A screen-negative result does not exclude the possibility of an affected pregnancy.

The integrated test can identify pregnancies at high risk of Edwards' syndrome (trisomy 18). In cases where the risk is high this is reported.

ACTION FOLLOWING A SCREEN-POSITIVE RESULT

If the result is screen-positive for Down's syndrome the women concerned are offered a diagnostic amniocentesis.

If the result is screen-positive for

open neural tube defects then a detailed ultrasound scan at about 18 to 20 weeks is offered and possibly an amniocentesis.

REPORTING OF RESULTS

The screening results are usually ready within 48 hours of receipt of the blood sample and will be sent to the antenatal clinic or doctor who ordered the test. Screen-positive results are telephoned and faxed directly to the antenatal clinic or doctor.

PERFORMANCE OF THE INTEGRATED TEST

The screening performance of a test is usually defined in terms of the detection rate (also called 'sensitivity'), false positive rate and the odds of being affected given a positive result (OAPR) which is the ratio of true positives to false positives. The detection rate (DR) is the proportion of affected pregnancies with screen-positive results and the false positive rate (FPR) is the proportion of unaffected pregnancies with screen-positive results.

Down's syndrome

DR = 90% for a 1.3% FPR

OAPR = 1:5 (i.e. among women with a screen-positive result for Down's syndrome one will have an affected pregnancy for every 5 that do not).



Open Neural Tube Defects

DR = 85% with open spina bifida (and nearly 100% with anencephaly) for about a 1% FPR.

OAPR = 1:15 (i.e. among women with a screen-positive result for open neural tube defects one will have an affected pregnancy for every 15 that do not).

CALCULATION OF THE RISK OF DOWN'S SYNDROME

Maternal age

The risk of having a pregnancy with Down's syndrome increases with maternal age as shown in table 1 below. The maternal age-specific risk is the background risk of Down's syndrome that is used to calculate a woman's screening result based on the measurement of the screening markers.

The markers

In the first trimester, the PAPP-A level is, on average, low in Down's syndrome pregnancies (about half that of unaffected pregnancies), and the NT measurement is, on average, high (about double that of unaffected pregnancies). In the second trimester AFP and uE_3 levels are, on average, low (about three-quarters that of unaffected pregnancies) and inhibin-A and free β -hCG levels are, on average, high (about double that of unaffected pregnancies).

The concentrations of the markers vary with gestational age. In the first trimester PAPP-A and nuchal translucency increase with gestational age. In the second trimester AFP and uE_3 increase, free β -hCG decreases, and inhibin-A decreases before

Table 1

Maternal age at EDD*	Risk of Down's syndrome ⁺	Maternal age at EDD*	Risk of Down's syndrome ⁺	Maternal age at EDD*	Risk of Down's syndrome ⁺
20	1:1450	30	1:940	40	1:85
21	1:1450	31	1:820	41	1:70
22	1:1450	32	1:700	42	1:55
23	1:1400	33	1:570	43	1:45
24	1:1400	34	1:460	44	1:40
25	1:1350	35	1:350	45	1:35
26	1:1300	36	1:270	46	1:30
27	1:1200	37	1:200	47	1:30
28	1:1150	38	1:150	48	1:30
29	1:1050	39	1:110	49	1:25

*EDD = expected date of delivery

Morris et al (2003)

⁺Ratio of affected to unaffected pregnancies

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17 weeks and increases after 17 weeks. Also, the measurement of serum markers may vary between laboratories. In order to take account of these sources of variation, the concentration of each marker is expressed as a multiple of the median for pregnancies of the same gestational age (MoM).

The figure below illustrates the concept. A hypothetical marker has a median level of 25 iu/mL at 10 weeks, 50 iu/mL at 12 weeks and 100 iu/mL at 14 weeks. If a woman were found to have a level of 50 iu/mL at 10 weeks her level would be twice the median (50/25) or 2.0 MoM. Similarly if the level were 50 iu/mL at 14 weeks this would be half the median (50/100) or 0.5 MoM.

Risk of Down's syndrome in relation to marker levels

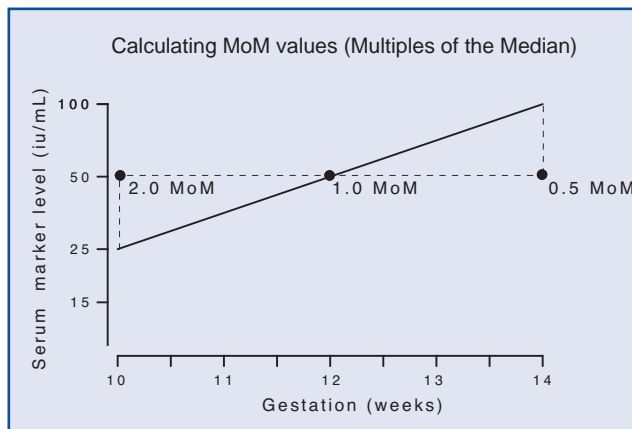
The graphs on the opposite page show the overlapping relative frequency distributions of the markers in Down's syndrome pregnancies and unaffected

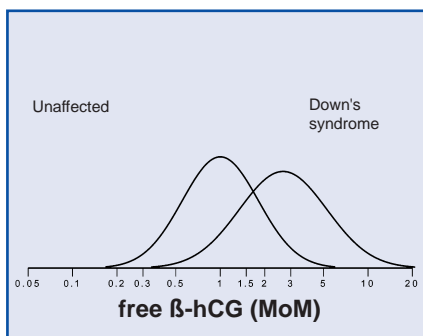
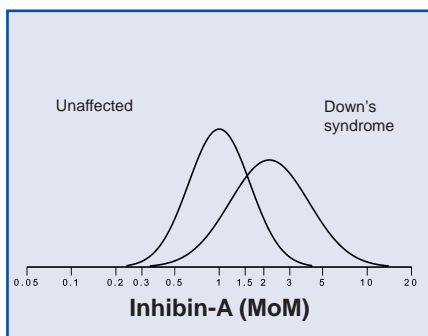
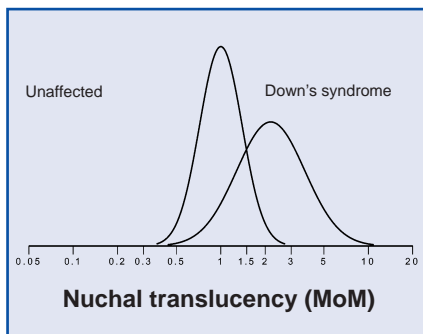
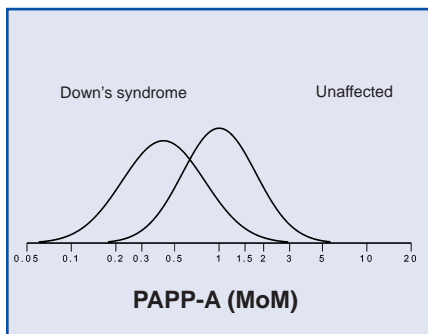
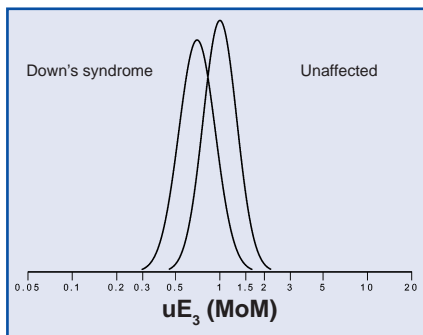
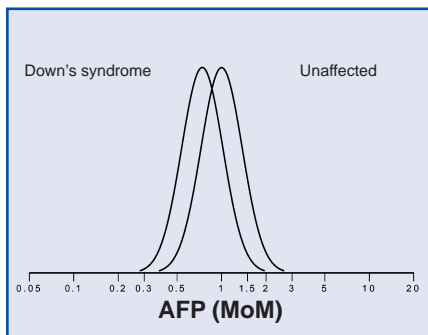
pregnancies. The points of intersection are the value at which the risk of Down's syndrome is the same as the background risk in the population. From these graphs, it can be seen that AFP, uE₃ and PAPP-A values below 0.86 MoM, 0.83 MoM and 0.64 MoM respectively and NT, inhibin-A and free β-hCG values above 1.46 MoM 1.54 MoM and 1.67 MoM respectively will tend to increase the risk of Down's syndrome above the background risk while values in the opposite directions will tend to decrease the risk below the background risk.

FACTORS AFFECTING THE TEST

Maternal weight, ethnic group , In Vitro Fertilisation (IVF), Insulin Dependent Diabetes Mellitus (IDDM) and smoking

- Serum marker levels tend to be decreased in heavier women, and increased in lighter women.





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- AFP levels tend to be about 20% higher, free β -hCG levels about 10% higher and PAPP-A levels about 60% higher in Afro-Caribbean women than in Caucasian women.
- Free β -hCG levels tend to be about 10% higher and uE_3 levels about 10% lower in women who have become pregnant as a result of IVF compared with non-IVF pregnancies
- AFP and uE_3 levels tend to be low (about 8% and 6% respectively) in women with insulin dependent diabetes mellitus.
- PAPP-A and free β -hCG levels tend to be about 20% lower and inhibin levels about 60% higher in women who smoke.

Appropriate adjustments of the MoM values are made for these factors.

Twins

The serum marker levels are raised in twin pregnancies. Adjustments are made to take account of this.

Screening in twin pregnancies poses a difficulty because of the possibility that one fetus may be affected and the other may not. Because of the presence of two fetuses the amniocentesis is a slightly more complex procedure in a twin pregnancy. If one twin is found to be affected and the other unaffected, selective feticide can be offered. This procedure poses a substantial risk to

the unaffected twin. The presence of a twin pregnancy may therefore be seen by some women as a reason to avoid screening.

Previous affected pregnancies

If a previous pregnancy with Down's syndrome or open neural tube defect is reported, the result will be classified as 'screen-positive' regardless of the level of the screening markers so that further testing can be discussed with the woman. A risk is calculated which takes account of a woman's previous pregnancy with Down's syndrome. The woman's age at the time of her previous pregnancy with Down's syndrome affects the recurrence risk and this is taken into account in the risk calculation.

Taking account of screening in a previous pregnancy

If a woman has been screened for Down's syndrome or open neural tube defects in a previous pregnancy the levels of the screening markers in that pregnancy can be used to adjust the marker levels in the current pregnancy. This is useful because markers used in screening tend to 'track' between pregnancy (e.g. a free β -hCG level that is high in one pregnancy tends to be high in a subsequent pregnancy). So a woman with a false positive result in one pregnancy is likely to have a false positive result again in a subsequent pregnancy. Adjusting marker levels for those in a previous pregnancy can help avoid this problem of false-positives recurring in different pregnancies.



Vaginal bleeding

Vaginal bleeding immediately before taking the second blood sample can affect the screening result by increasing the maternal serum AFP level and so, in these circumstances, it may be advisable to delay collecting blood for the screening test until a week after bleeding has stopped.

Testing after amniocentesis

If an amniocentesis has been attempted in the pregnancy prior to taking the second blood sample, the result cannot be interpreted. This is due to the possibility of feto-maternal transfusion which can increase the maternal serum AFP level.

EFFECT OF MATERNAL AGE ON SCREENING PERFORMANCE

An older woman is more likely to have a screen-positive result than a younger woman as she starts with a higher age-specific risk of Down's syndrome. For this reason, the test is more likely to detect a Down's syndrome pregnancy in an older woman than in a younger woman.

Table 2 below shows, according to age, the probability of a screen-positive result and the proportion of Down's syndrome pregnancies detected. Whatever the woman's age, the best estimate of her risk of having an affected pregnancy is obtained using her age in conjunction with her marker values.

Table 2

Maternal age group (years)	Probability of a screen-positive result	Proportion of Down's syndrome pregnancies detected (%)
Under 25	1 in 100	85
25-29	1 in 90	86
30-34	1 in 50	89
35-39	1 in 20	94
40-44	1 in 8	97
45 and over	1 in 5	98
All	1 in 65	90

(early mid-trimester estimates, first stage performed at 11 completed weeks of pregnancy)

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COMPARISON WITH OTHER DOWN'S SYNDROME SCREENING TESTS

Table 3 below shows the estimated detection rate (DR) and odds of being affected given a positive result (OAPR) for various Down's syndrome screening methods using a 5% fixed false positive rate (FPR) and, for

the integrated test, also using a 1% false positive rate. The estimates are based on a large UK study (Wald et al 2003) and apply to the early second trimester of pregnancy. They are corroborated by results from other studies.

Table 3

Method of screening	FPR(%)	DR (%)	OAPR
Maternal age alone	5	32	1:56
Triple test (AFP, uE ₃ , free β-hCG)	5	78	1:23
Quadruple test (AFP, uE ₃ , free β-hCG, inhibin)	5	83	1:21
Combined test (Nuchal translucency [NT], free β-hCG, PAPP-A at 11 weeks)	5	89	1:20
Serum Integrated test (PAPP-A at 11 weeks and Quadruple markers at 14-22 weeks)	5	87	1:20
Integrated test (NT and PAPP-A at 11 weeks and Quadruple markers at 14-22 weeks)	5	95	1:19
	1	89	1:4

(Gestational age estimated by ultrasound scan and marker levels adjusted for maternal weight)
NB All tests include maternal age



DIAGNOSTIC TESTS

Amniocentesis

An amniocentesis is performed at about 15 to 16 weeks of pregnancy. Under ultrasound guidance a sample of amniotic fluid is collected using a needle inserted through the abdominal wall. Cells from the sample can be used to diagnose Down's syndrome. The risk of miscarriage due to the procedure is about 1%.

Down's syndrome is diagnosed using a technique called quantitative fluorescence polymerase chain reaction (QF-PCR). This provides a rapid diagnosis of Down's syndrome, usually within 48 hours of the amniocentesis being performed. It also detects trisomy 18, 13 and sometimes sex chromosome abnormalities. To diagnose other conditions the cells must grow before they can be examined and so the final results can take up to 2-3 weeks.

Chorionic Villus Sampling (CVS)

Occasionally this test may be offered as an alternative to amniocentesis. CVS involves taking a sample of placental tissue, by inserting a needle through the abdominal wall or a fine instrument through the cervix. As with amniocentesis QF-PCR is used to provide a rapid diagnosis for Down's syndrome, trisomy 18 and 13 and sometimes sex chromosome abnormalities. At this stage of pregnancy the risk of miscarriage due to the procedure is thought to be about the same as the risk following an amniocentesis.

With CVS there is a chance (about 1 in 100) that the test will not provide a conclusive result. In these circumstances an amniocentesis will need to be performed to provide a definite diagnosis.

Detailed ultrasound scan

This is offered when women are reported as screen-positive due to an increased risk of an open neural tube defect. Nearly all cases of anencephaly and open spina bifida can be detected.

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PATIENT INFORMATION

Points to remember when discussing the screening test with a woman considering whether to be screened:

- Obtain an explicit decision on whether to be screened.
- Assess her knowledge of Down's syndrome and whether more information is needed.
- Satisfy yourself that she understands that the test does not give a definitive answer – it divides women into a higher risk group (screen-positive) and a lower risk group (screen-negative). For Down's syndrome the result is screen-positive if the risk is 1 in 150 or greater. For open neural tube defects the result is screen-positive if the AFP level is 2.5 MoMs or higher.
- Explain that about 1 in 65 women screened will have a screen-positive result for Down's syndrome and they will be offered an amniocentesis or a CVS, both of which carry a risk of miscarriage. Most women with a screen-positive result will **not** have affected pregnancies.
- Check that she knows that the test will not detect all pregnancies with Down's syndrome.
- Explain that in the few pregnancies in which Down's syndrome is diagnosed, the woman will be offered a termination of pregnancy.

Women should have the opportunity to have time to consider whether to be screened, and discuss this with others before making a decision. While screening cannot provide complete reassurance and will cause anxiety, particularly if the screening test is positive, it provides the opportunity of finding out whether the pregnancy is affected with Down's syndrome. If women do not want this information while pregnant screening is best avoided.

USEFUL TELEPHONE NUMBERS

Antenatal Screening Service, Barts and The London School of Medicine and Dentistry.....	020 7882 6293
Down's Syndrome Association.....	020 8682 4001
Association for Spina Bifida and Hydrocephalus.....	01733 555988
Antenatal Results and Choices (ARC).....	020 7631 0285



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Antenatal Screening

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The Wolfson Institute of Preventive Medicine has played a leading role in the discovery, development and implementation of antenatal screening methods. It is committed to improving the efficacy and safety of screening. We use information collected as part of our screening programme, including measurements on stored blood samples, to audit our screening programme and ensure that it is meeting our expected quality standards. Such information may also be used to help discover and validate new tests that improve the quality of screening services.