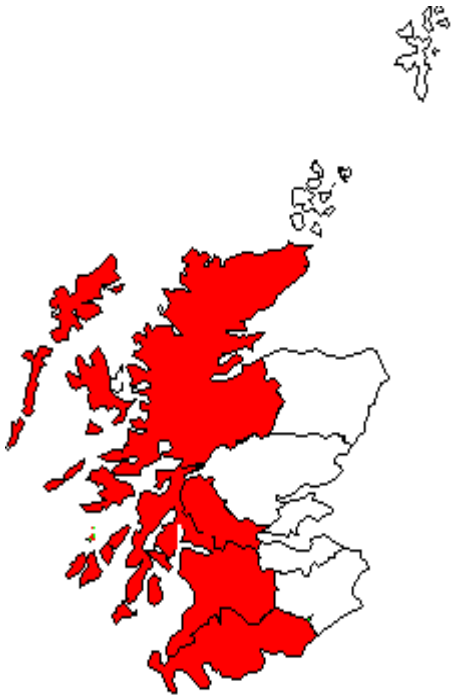
NHS SCOTLAND

WEST OF SCOTLAND REGIONAL PREGNANCY SCREENING SERVICE



Information and Guidelines for Health Professionals

V5 October 2015

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General Information

- Phone number
  Prenatal Screening Office: 0141 354 9272

- Address
  Prenatal Screening Laboratory
  West of Scotland Genetic Services
  Level 2, Laboratory Medicine
  Queen Elizabeth University Hospital
  1345 Govan Road
  Glasgow
  G51 4TF

- Laboratory Hours
  Weekdays between 0900-1700

- Delivery of samples to laboratory
  Samples should be sent to the laboratory as soon as possible after collection. If sending by post, use First Class mail and ensure that the packaging is rigid and conforms to current postal regulations.

- Danger of Infection
  Health and Safety law demands that those who need to know (e.g. laboratory staff) are alerted to an identified risk of infection. This enables appropriate laboratory procedures to be used to minimise risk. It is the responsibility of the person taking the sample from a patient known to have, or be at risk of, an infectious disease, to ensure that both the form and the specimen container are appropriately labelled.

Screening tests

Two different screening tests are currently available from the laboratory:

- First trimester Combined Ultrasound and Biochemical screening for Down's syndrome (11+2 weeks to 14+1 weeks gestation). Green request form

- Second trimester screening for Down's syndrome (14+2 weeks to 20 weeks of gestation). White request form.

Sample

- 5-10mls of clotted blood are required. Please use tube with NO anticoagulant or other additive.

- Blood samples contaminated with EDTA or fluoride-oxalate are unsuitable for analysis as these affect the assays used and cause inappropriately low results for some markers. Never decant blood from EDTA or fluoride-oxalate tubes into other

tubes. To avoid the possibility of contamination, blood samples for prenatal screening should always be collected before any other samples.

- The sample tube must be sealed inside the plastic bag attached to the request form.

- The sample tube must be labelled. Unlabelled samples will not be accepted for analysis.

- Samples must be sent to the laboratory quickly. Samples more than **3 days old on receipt for first trimester**, (**5 days for second trimester screening**) will be unsuitable for analysis and a repeat will be requested. Please plan carefully for weekends and Public Holidays.

- If samples need to be stored for a short time before dispatch to the laboratory, store in cool conditions, but not frozen. Freezing renders samples unsuitable for analysis.

Request form

- Please complete the request form in full. If incorrect information is supplied or information is missing then the interpretation of the results may be invalid.

- See the reverse side of the request form for further information on completion of the form.

Patient information leaflets

- Information leaflets about pregnancy screening are available from your local Health Information Department. An electronic version and translations into other languages are available from the Health Scotland website (www.healthscotland.com).

Health Boards Served

The Glasgow Prenatal Screening Service covers the following Health Boards:
Ayrshire and Arran
Dumfries and Galloway
Greater Glasgow and Clyde
Forth Valley
Highland
Lanarkshire
Western Isles

Laboratory Contacts

The Laboratory Prenatal Screening Co-ordinator is:

Ms Louise Brown
Principal Clinical Scientist
Tel: 0141 354 9274
E-mail louise.brown@ggc.scot.nhs.uk

The Head of Service for Genetics is:

Mr Gordon Lowther
Consultant Clinical Scientist
Tel: 0141 354 9297
E-mail gordon.lowther@ggc.scot.nhs.uk

INTRODUCTION

Overview of screening

All pregnant women in Scotland are offered screening for trisomy 21 (Down's syndrome) in early pregnancy which provides them with information, allowing them to make informed choices.

Screening is a two stage process. The primary screening test provides a risk (i.e. "chance" or "probability") that the fetus is affected by a chromosomal abnormality. A second, diagnostic test is offered to those women who have a higher chance that their pregnancy is affected, and this provides a definitive result.

Patient choices

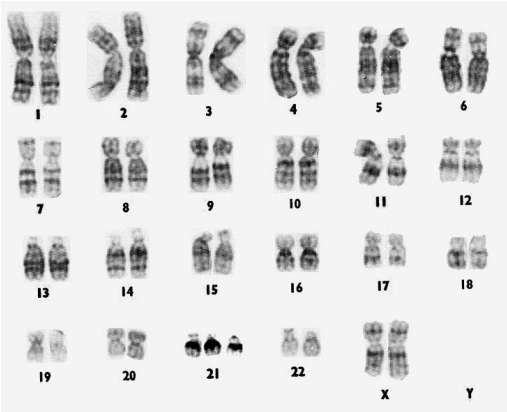
The tests are "opt-in", women may choose to be screened or not to be screened and may opt out of the process at any stage. Women should receive an explanatory leaflet in advance of the date of their antenatal clinic appointment so that they have time to consider the implications of accepting or declining testing and can ask further questions about the tests. Important issues to bear in mind when counselling women are that pregnancy screening tests do not detect all affected fetuses and a proportion of affected cases are missed (i.e. false negative results). Also, for those pregnancies which are defined as "screen positive" (i.e."Higher chance") by the test, most are in fact falsely positive, and most women in this group will have normal healthy babies. However, all women in this "higher chance" group require further counselling and are offered further testing which will provide a definitive diagnosis and this process can be a cause of significant anxiety. Where an affected fetus is diagnosed, women have the option to continue with or terminate the pregnancy and it is important that these women are offered help and support at this time.

Screening for Down's Syndrome may be carried out either in the first or second trimester. First trimester combined screening is the optimum method for screening for Down's Syndrome as documented in the current UK National Screening Committee Model of Best Practice.

More detailed information on Down's screening may be obtained from http://www.pnsd.scot.nhs.uk/wp-content//Fetal-Anomaly-and-Downs-Syndrome-Screening-Protocols-version-2.0.pdf

DOWN'S SYNDROME (Trisomy 21)

People with Down's syndrome (trisomy 21) have an extra chromosome, having three copies of chromosome 21 instead of two. Chromosomes carry genes which pass certain characteristics from parents to their children. Abnormalities can occur when there are too many chromosomes. In the majority of cases, the extra chromosome 21 is of maternal origin. About 95% of cases of Down's syndrome are regular trisomy 21 and about 4% are the result of an inherited maternal or paternal balanced translocation (often 14;21). For this reason, in the case of a previous affected pregnancy or a positive family history of Down's syndrome the parental chromosomes should be analysed for evidence of a translocation. About 1% of Down's syndrome cases are mosaic, having both normal (two chromosomes 21) and trisomic (three chromosomes 21) cell lines.



Without the intervention of screening and prenatal diagnosis the incidence of Down's syndrome in the Scottish population would be around 1 in 700 births. The exact population incidence is dependant on the age structure of the pregnant population as older mothers are more likely to have a baby with Down's syndrome. The table below shows the age related chance of having an affected birth for different maternal ages.

Table 1: Age related chance of having a Down's syndrome pregnancy at birth.

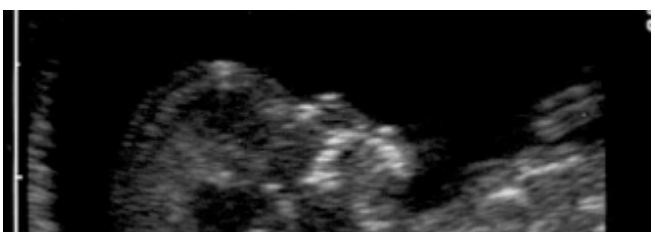
Maternal age (years)	Chance of a Down's syndrome birth	Maternal age (years)	Chance of a Down's syndrome birth
16	1 in 1572	31	1 in 796
17	1 in 1562	32	1 in 683
18	1 in 1556	33	1 in 574
19	1 in 1544	34	1 in 474
20	1 in 1528	35	1 in 384
21	1 in 1507	36	1 in 307
22	1 in 1481	37	1 in 242
23	1 in 1447	38	1 in 189
24	1 in 1404	39	1 in 146
25	1 in 1351	40	1 in 112
26	1 in 1286	41	1 in 85
27	1 in 1208	42	1 in 65
28	1 in 1119	43	1 in 49
29	1 in 1018	44	1 in 37
30	1 in 909	45	1 in 28

FIRST TRIMESTER COMBINED ULTRASOUND AND BIOCHEMICAL SCREENING

Test principle

Combined Ultrasound and Biochemical screening uses a combination of ultrasound measurements of fetal nuchal translucency (NT), measurement of maternal serum markers free beta hCG (FβhCG) and pregnancy associated plasma protein A (PAPP-A) to derive a combined risk for Down's syndrome. Each of these markers, including NT, varies with gestation and an accurate measurement of fetal maturity is required for accurate interpretation of results. For first trimester screening, ultrasound measurement of fetal crown rump length (CRL), carried out at the same time as the NT measurement, is used as the basis of the calculation of gestation for conversion of marker levels into a multiple of the median (MoM). When, in exceptional circumstances, the sample is taken on a different day from the NT measurement the gestation estimated at the time of NT measurement is also used as the basis for the gestation at sampling, after allowing for the time difference in days.

NT is the fluid filled area which is present at the back of the fetal neck in all pregnancies and measures around 1.0mm in unaffected pregnancies at 11-13 weeks gestation. This area tends to be enlarged when there is a fetal chromosome abnormality present and the NT size can be accurately measured by ultrasound (to the nearest 0.1mm) following a defined protocol and using high resolution equipment. In Down's syndrome pregnancies NT size is increased in the majority of pregnancies to about double the size in unaffected pregnancies of equivalent gestation. The NT measurement is converted to a multiple of the median NT size (MoM) at the appropriate CRL and a risk estimated.



In the first trimester, the maternal serum concentration of the biochemical marker FβhCG is increased in Down's syndrome pregnancies to levels approaching twice those in unaffected pregnancies (2.0 MoM). PAPP-A levels are reduced in affected pregnancies to around half the normal level (0.5 MoM) although the magnitude of the change is related to the gestation being greatest at earlier gestations. A combined risk of Down's syndrome is derived from (1) the NT measurement in MoM, (2) the FβhCG MoM, (3) the PAPP-A MoM and (4) the maternal age risk.

First trimester combined screening

The definition of whether a pregnancy is "low chance" or "higher chance" on combined screening is based on an odds ratio of 1 in 150. All screening risk results equal to or greater than 1 in 150 are "higher chance" and these women are offered further counselling and diagnostic testing. About 2% of women having combined screening receive a "higher chance" result. All screening risk results less than 1 in 150 are "low chance" and no further action is usually indicated in these cases.

Most women in the "higher chance" group following combined screening will not have an affected pregnancy (i.e. these are false positive screening results).

Women in the "low chance" group following combined screening may still have an affected pregnancy, with the likelihood being indicated by the risk given on the report (e.g. 1 chance in 380).
It is important to note that, because maternal age is a component of the screening risk calculation, the test detects an increasing proportion of Down's syndrome pregnancies as maternal age advances (see Table 2) and a greater proportion of older women are assigned to the 'Higher chance' group.

Table 2: Detection rates for Down's syndrome and corresponding false positive rates in first trimester in different maternal age groups

Age group (years)	Detection rate for Down's syndrome (%)	False positive rate (%)
<20	73	1.1
25	74	1.2
30	78	1.8
35	85	3.8
40	92	10.8
45	97	29.6

Multistage testing

It is important to note that women who have had first trimester combined screening should **not** subsequently go on to have a second trimester biochemical screening test for Down's syndrome.

NT MEASUREMENT AND SAMPLES REQUIRED FOR FIRST TRIMESTER COMBINED SCREENING

For combined screening, NT measurements should be taken only when the fetal CRL falls within the range of 45-84 mm (equivalent to 11+2–14+1 weeks of gestation). Before this the fetus is too small to allow accurate NT measurement and after 14 weeks, the strength of the association between NT and fetal chromosome abnormalities begins to decline. It is best practice to take a measurement from more than one image. The maximum NT measurement that meets all criteria should be used for the risk calculation. Measurement of NT should only be carried out by sonographers after appropriate training.

Blood samples should be taken by venepuncture, usually at the time of the NT measurement (CRL 45-84mm). For satisfactory laboratory testing, 5-10 mls of clotted blood are required. The sample tube should be closed securely to guard against leakage and sealed inside the plastic bag that is attached to the request form before despatch to the laboratory.

Please use the **green** request form for first trimester screening.

FIRST TRIMESTER SCREENING IN MULTIPLE PREGNANCIES

Currently, ultrasound NT measurement alone offers a practical method of determining risk as NT measurements in each twin/ triplet fall within the same range as those in unaffected singleton pregnancies. In twin pregnancies where one (or both) fetuses are affected by Down's syndrome, NT size is increased in the affected fetus(es) and a risk of Down's syndrome can be calculated from the individual NT measurement in each fetus, combined with the maternal age risk.

A separate risk is reported for each fetus.

REPORTING OF COMBINED SCREENING RESULTS

Using a 1 in 150 threshold risk to define the "higher chance" group, around 2% of women will be classified as "higher chance of Down's syndrome". As these results require further action, they are EMAILed as soon as they are available to the referring source so that the patient may be recalled for counselling as quickly as possible. Hard copies of the results (both higher chance and low chance) are sent by post for filing in the patient record.

It is important that combined screening reports received in the antenatal clinic are checked for accuracy of information before being filed. This includes gestation, date of birth, maternal weight, smoking status, ethnicity and any other details which might affect interpretation of results. If anything is found to be incorrect then the laboratory should be contacted immediately so that a revised report can be issued. It is important that all patients receive notification of their result – whether higher chance or low chance.

DIAGNOSTIC TESTS

Women whose first trimester screening results give a higher chance of a chromosome abnormality and who wish a diagnostic test are offered chorionic villus sampling which in the first trimester carries a procedural miscarriage rate of about 1-2%.

It is a woman's choice whether she decides to have a diagnostic test after a higher chance result.

SCREENING FOR DOWN'S SYNDROME IN THE SECOND TRIMESTER

Women who wish to be screened should be encouraged to do so in the first trimester as it provides a higher detection rate and lower false positive rate than second trimester screening. The second trimester screen does however provide a screening option for women who present too late for first trimester screening.

The current second trimester screening test ultilises measurement of maternal serum alpha fetoprotein (AFP), human chorionic gonadotrophin (hCG), unconjugated oestriol (UE3) and Inhibin A. Pregnancies affected by Down's syndrome have elevated levels of hCG and Inhibin A (on average around twice the levels found in unaffected pregnancies at 2.06 MoM and 1.99 MoM respectively), and lower levels of AFP and UE3 (on average around 75% of those in unaffected pregnancies at 0.75 MoM and 0.72 MoM respectively). The risk of Down's syndrome is derived from

- the AFP MoM
- the hCG MoM
- the UE3 MoM
- the Inhibin A MoM
- and the maternal age risk.

As with first trimester screening, the definition of whether a pregnancy is "low chance" or "higher chance" is based on an odds ratio of 1 in 150. All screening risk results equal to or greater than 1 in 150 are "higher chance" and these women are offered further counselling and diagnostic testing.

Using a cut-off odds ratio of 1 in 150, around 3-4% of pregnancies will be 'higher chance'.

It is important to note that as with first trimester screening, maternal age is a component of the screening risk calculation, and likewise the test detects an increasing proportion of Down's syndrome pregnancies as maternal age advances (see Table 3) and a greater proportion of older women are assigned to the 'Higher chance' group.

Table 3: Detection rates for Down's syndrome and corresponding false positive rates in second trimester in different maternal age groups

Age group (years)	Detection rate for Down's syndrome (%)	False positive rate (%)
<20	57	1.5
25	58	1.8
30	64	2.8
35	75	6.6
40	88	19.7
45	97	50.7

When AFP concentration is elevated – in view of the increased chance of neural tube defects and other structural anomalies, this will be reported as a higher chance result. An elevated result is defined as an AFP concentration equal to or greater than 2.0 multiples of the median (MoM) i.e. twice that in unaffected pregnancies.

It should be noted that women who have been successfully screened for Down's syndrome in the first trimester will not be screened using AFP only to produce a risk of structural anomalies. Any anomalies should be detected by the fetal anomaly ultrasound scan offered to all women between 18+0 and 20+6 weeks gestation.

REPORTING OF SECOND TRIMESTER RESULTS

Using a 1 in 150 threshold risk to define the "higher chance" group, these results require further action, they are EMAILed as soon as they are available to the referring source so that the patient may be recalled for counselling as quickly as possible. Hard copies of the results (both higher chance and low chance) are sent by post for filing in the patient record.

As with first trimester results, it is important that screening reports received in the antenatal clinic are checked for accuracy of information before being filed. This includes gestation, date of birth, maternal weight, smoking status, ethnicity and any other details which might affect interpretation of results. If anything is found to be incorrect then the laboratory should be contacted immediately so that a revised report can be issued. It is important that all patients receive notification of their result – whether higher chance or low chance.

DIAGNOSTIC TESTS

Women with a higher chance of Down's syndrome should be offered amniocentesis in order to exclude or identify a chromosome abnormality. Amniocentesis is associated with a risk of miscarriage of around 1%.

It is a woman's choice whether she decides to have a diagnostic test after a higher chance result.

SAMPLES REQUIRED FOR SECOND TRIMESTER SCREENING TEST

Blood samples should be taken by venepuncture between 14+2 weeks and 20+0 weeks of pregnancy. For satisfactory laboratory testing, 5-10 mls of clotted blood are required. The sample tube should be closed securely to guard against leakage and sealed inside the plastic bag that is attached to the request form before despatch to the laboratory.

Please use the **white** request form for second trimester screening for Down's syndrome.

MATERNAL AND PREGNANCY FACTORS AFFECTING INTERPRETATION OF SERUM MARKER CONCENTRATION

Several factors have been identified which affect serum marker concentrations and therefore the risk estimate derived from them. Corrections, to take account of these variables, can be made to provide a more accurate estimate of risk for individual women.

Gestation

All serum marker concentrations vary with gestation. Interpretation of marker concentration and NT measurements to take account of gestational variation is achieved by expressing results as a multiple of the appropriate gestational median level in unaffected pregnancies, but the precision of this estimate depends on the accuracy of the gestational estimate. Screening results cannot be interpreted without an accurate estimate of gestation.

In line with current UK guidelines we use an ultrasound estimate of gestation in preference to that calculated from LMP. The request forms ask for the CRL or HC and the date these were measured. A gestation will be calculated from these using standard formulae. The EDD is also requested, and this is used as an internal check on the calculated gestation.

The formulae used to calculate gestation are:

From CRL in mm:

Gestation in days = ((Sqrt((CRL + 1) * 1.037) * 8.052) + 23.73)

Robinson HP, Fleming JE (1975). *Br J Obstet Gynaecol*; **82**:702-10.

From HC in mm

Gestation in days = $\exp(3.7939 + 0.00611*HC - 0.000030321*HC^2 + 0.000000043498*HC^3)$

Altman DG, Chitty LS (1997). *Ultrasound Obstet Gynecol*, **10**, 174-91

Maternal weight
Women with higher than average weight (i.e. greater than around 67 Kg) will tend to have an increased blood volume and this results in a dilution of the concentration of the screening markers secreted into the maternal circulation. This will result in lower than average serum marker concentrations which will in turn affect the accuracy of the final calculated risk. The opposite effect is found in women of lower than average maternal weight.

The effect of maternal weight is particularly marked at the extremes of the weight range and a correction can be applied which ensures that there is no over- or under- representation of very large or very small women within the higher chance groups.

Maternal weight has no effect on NT measurements.

Maternal smoking
Smoking in pregnancy is known to affect the normal functioning of the placenta and in the second trimester this is reflected in reduced hCG and UE3 secretion compared to non-smokers. AFP and Inhibin A concentrations are also known to be increased.

In the first trimester PAPP-A concentration is reduced to around 81% of the levels found in non-smokers and this leads to a reduced detection rate for Down's syndrome if results are left uncorrected.

Correction for smoking status gives all women a more accurate risk.

Twin pregnancies
For screening twin pregnancies first trimester using NT measurement and maternal age alone is the current approach as discussed above. Evidence for screening in the second trimester in twin pregnancies is currently being reviewed but is not currently offered as an option.

Assisted reproduction
An important practical consideration in IVF pregnancies is that the age of an egg donor (if applicable) must be used to derive the maternal age risk, while for frozen embryos, the age at conception should be used.

There is currently insufficient data to demonstrate any changes in concentration for the serum markers used in first trimester screening.

Previous affected pregnancy: Down's syndrome
The risk of a recurrence of Down's syndrome is increased where a woman has had a previously affected pregnancy. A residual risk of 0.34% at term has been calculated and is additional to the maternal age risk. This sharply increases the likelihood that a screening result will be "higher chance" in women with a previous affected pregnancy, especially in younger women. Table 4 shows the population maternal age risks modified to take a previous Down's syndrome pregnancy into account.

Table 4: Age related chance of having a Down's syndrome pregnancy at birth in women who have had a previous pregnancy affected by Down's syndrome.

Maternal age (years)	Chance of a Down's syndrome birth (previous affected pregnancy)	Maternal age (years)	Chance of a Down's syndrome birth (previous affected pregnancy)
16	1 in 248	31	1 in 215
17	1 in 248	32	1 in 206
18	1 in 247	33	1 in 194
19	1 in 247	34	1 in 181
20	1 in 247	35	1 in 167
21	1 in 246	36	1 in 150
22	1 in 245	37	1 in 133
23	1 in 244	38	1 in 115
24	1 in 243	39	1 in 97
25	1 in 242	40	1 in 81
26	1 in 239	41	1 in 66
27	1 in 237	42	1 in 53
28	1 in 233	43	1 in 42
29	1 in 228	44	1 in 33
30	1 in 222	45	1 in 25

For a 14;21 translocation Down's syndrome the risk of recurrence is at least 1 in 10 if the woman is a carrier and about 1 in 20 if her partner is a carrier. In this situation screening is not appropriate and the couple should consider invasive prenatal diagnosis. For a 21;21 translocation in either parent the risk is 100% and a naturally conceived normal pregnancy is not possible.

Other factors

Ancestry/ Ethnicity

Information on ancestry based on the family origin questionnaire is requested. This information allows the appropriate ethnic median concentration to be used to calculate the MoM and produce a more accurate risk estimate. Please see the back of the laboratory request form for more information.

Vaginal bleeding

Vaginal bleeding in the two weeks preceding the screening sample being taken may cause a significant rise in maternal serum AFP concentration. It is not thought to affect other markers.