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BIOCHEMICAL SCREENING FOR FETAL CHROMOSOME ABNORMALITIES

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Thesis submitted to

FACULTY OF MEDICINE

UNIVERSITY OF GLASGOW

for the degree of

DOCTOR OF PHILOSOPHY

November 1993

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To

DAVID

and to my children

GILLIAN AND DAVID

I cannot rest from travel: I will drink
Life to the lees: all times I have enjoyed
Greatly, have suffered greatly, both with those
That loved me, and alone; on shore, and when
Through scudding drifts the rainy Hyades
Vext the dim sea: I am become a name;
For always roaming with a hungry heart
Much have I seen and Known; cities of men
And manners, climates, councils, governments,
Myself not least, but honoured by them all;
And drunk delight of battle with my peers,
Far on the ringing plains of windy Troy.

I am a part of all that I have met;
Yet all experience is an arch wherethrough
Gleams that untravelled world, whose margin fades
For ever and forever when I move.

Ulysses
Alfred, Lord Tennyson
1809-1892

DECLARATION

Excerpts from the results presented in this thesis have been published as detailed on pages 20-22. I certify that this thesis does not contain any other material published or written by any other person except where due reference is made in the text. The results presented in this thesis have not been submitted for any other degree or diploma.

JENNIFER A CROSSLEY

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ACKNOWLEDGEMENTS

I would like to thank the following people:

Professor JM Connor for providing me with the opportunity to undertake this project, and for his encouragement and guidance throughout the course of this study.

Dr David Aitken for supervising this project and for much encouragement and help throughout the six years it has taken to complete this thesis.

My colleagues and friends, both past and present, in the Biochemical Genetics Division, David Aitken, Esther Berry, David Farquharson, Elspeth Gracey, Gordon Graham, Gary McCaw, Karen McInnes, Linda MacNeill, Liz Moon, Aileen Mutch, David Stevenson, Alison Stewart and Sheila Taylor, for their help and encouragement. None of this work would have been possible without 'The Team'.

Antenatal clinic, secretarial and medical records staff for their help in supplying casenotes and follow-up information on patients who had a chromosomally abnormal pregnancy.

Paul Batstone and Dr Anne Robb, Cytogenetics Department, Royal Nothern Infirmary, Inverness, for providing information on chromosomally abnormal pregnancies and births in Highland Region.

Esther Berry for much help with the follow up study on

routine hCG/AFP/age screening.

Gordon Graham for help with computing.

My children Gillian and David for putting up with the fact that I have often had too much 'homework'.

Initial data collection and preliminary analysis on AFP levels in autosomal trisomy pregnancies was carried out by Dr Moshe Zeitune.

SP1 immunoelectrophoresis and compilation of results were carried out by Gordon Graham.

This work was partially supported by a grant from the Scottish Home and Health Department to Dr D Aitken and Professor JM Connor

PUBLICATIONS

Excerpts from the results presented in this thesis have been published or presented at scientific meetings as detailed below.

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ABREVIATIONS

Abbreviation	Meaning
16α-DHEAS	16α-hydroxy-dehydroepiandrosterone sulphate
AFP	Alphafetoprotein
BSA	Bovine seum albumin
CI	Confidence interval
cpm	Counts per minute
CV	Coefficient of variation
DHEAS	Dehydroepiandrosterone sulphate
Frβ-hCG	Free β subunit of human chorionic gonadotrophin
g	Grams
hCG	Human chorionic gonadotrophin
IU	International units
IRMA	Immunoradiometric assay
IEP	Immunoelectrophoreis
IRP	International reference preparation
K-S	Kolmogorov-Smirnov test
KU	Kilo units
l	Litre
mg	Milligrams
ml	Millilitre
nmol	Nanomole
MOM	Multiple of the median
mIU	Milli-international unit
n	Number
nmol	Nanomole
p	Probability
PBS	Phosphate buffered saline
r	Correlation coefficient

RIA	Radioimmunoassay
SD	Standard deviation
SP_1	Pregnancy-specific ß-1 glycoprotein
UE3	Unconjugated estriol
v/v	Volume to volume
w/v	Weight to volume
$\bar{x}$	Mean
µg	Microgram
µl	Microlitre

SUMMARY

The aim of this project was to explore ways of using biochemical screening to improve the prenatal detection rate for Down's syndrome and other chromosome abnormalities over that being achieved using maternal age alone. It is well established that the risk of a pregnancy being affected by Down's syndrome or one of the other autosomal trisomies increases with advancing maternal age, and since the early 1970s this has been the criterion used to select women for diagnostic fetal chromosome analysis. However, the majority of affected pregnancies (70%) are born to women under 35 years of age and the uptake of diagnostic testing amongst women aged 35 years and over has been relatively low (<40%) leading to only around 12% of the affected pregnancies being detected in practice.

The first phase of this study was a retrospective analysis of maternal serum alphafetoprotein (AFP) results from the west of Scotland prenatal screening programme for neural tube defects. This was carried out on 142 pregnancies with autosomal trisomy (114 Down's syndrome, 19 trisomy 18 and 9 trisomy 13) and 113,000 unaffected pregnancies screened at 16-20 weeks gestation between April 1982 and May 1987. Maternal serum AFP levels in the affected pregnancies were significantly reduced ($p<0.001$) to 0.72 multiples of the median (MOM) of the unaffected pregnancies. Risks (likelihood ratios) were derived from the overlapping log Gaussian distributions of the abnormal and unaffected pregnancies and combined with maternal age risks to give the overall odds of an affected pregnancy. Using a mid-trimester threshold risk of 1:280,

which approximates to the maternal age risk at age 35, an overall detection rate of 37% was predicted for a false positive rate of 6.6%.

Using data from the above study, the west of Scotland AFP screening programme for neural tube defects was adapted in 1987 to include reporting of the risk of an autosomal trisomy in individual pregnancies. Analysis of this routine screening in over 100,000 pregnancies has shown an improved detection of autosomal trisomies (43%) at a false positive rate of 6.1%. The uptake of diagnostic testing amongst the high risk group of women was relatively low (42%) especially amongst the women under 35 years (32%). However, the uptake of diagnostic testing in the women over 35 years assigned to the high risk group increased to 56% compared with that from maternal age alone of less than 40%. Overall, the prenatal diagnosis rate for autosomal trisomies was 25% in the screened population.

Although screening based on a combination of AFP levels and maternal age is more effective than that achievable with maternal age alone, around 60% of affected pregnancies are still not detected, mainly in women under 35 years. Greater sensitivity is potentially possible using additional information obtainable from the analysis of other pregnancy markers in maternal serum.

The second phase of this study was the retrospective analysis of four other pregnancy markers, human chorionic gonadotrophin (hCG), unconjugated estriol (UE3), pregnancy specific β_1-glycoprotein (SP_1) and the free β subunit of hCG (Frβ-hCG), by immunoassay, in

stored maternal serum samples from different types of chromosomally abnormal pregnancies. The objective was to define the level of variation associated with these analytes, to find the best combination of markers for routine clinical use, and to compare different approaches for combining risks from these markers.

Sixty-five thousand serum samples, collected prospectively in the west of Scotland between January 1987 and March 1989 at 15-20 weeks gestation for routine AFP estimation provided the source material for this study. After AFP estimation the samples were stored at -20°C until the outcome of the pregnancy was known. Subsequently 78 chromosomally abnormal pregnancies were identified for which stored serum was available. The abnormal cases consisted of 49 Down's syndrome, four trisomy 18, four trisomy 13, eight unbalanced translocations, four balanced translocations and nine sex chromosome abnormalities. Five control sera were selected for each case, matched for maternal age, gestation and time in frozen storage. Additional cases of Down's syndrome and trisomy 18 were added as they became available.

Intact molecule hCG levels were found to be significantly increased ($p<0.001$) in Down's syndrome pregnancies, with a median value of 2.18 MOM of the unaffected pregnancies, and significantly reduced in trisomy 18 pregnancies (0.21 MOM, $p<0.001$). UE3 levels were found to be significantly decreased with a median of 0.79 MOM of the controls in Down's syndrome ($p<0.02$) and 0.38 MOM in trisomy 18 ($p<0.01$). SP_1 levels were significantly increased ($p<0.01$) in Down's syndrome, with a median value of 1.17 MOM of

the controls, unchanged in trisomy 18 and significantly reduced in unbalanced translocations (0.52 MOM, $p<0.01$). Free ß subunit hCG levels were significantly increased in Down's syndrome with a median of 2.30 MOM of the controls ($p<0.001$) and reduced in trisomy 18 (0.23 MOM, $p<0.01$).

From the data derived from this retrospective study, the most effective combination of analytes for screening for Down's syndrome was found to be AFP and hCG, combined with maternal age. This combination gave a predicted 57% detection rate for Down's syndrome at a 5% false positive rate. UE3 was found to be less useful due to the high level of correlation found between it and AFP and using UE3 in addition to AFP and hCG reduced the predicted detection rate for Down's syndrome to 53% at a 5% false positive rate. SP_1 levels in Down's pregnancies showed only a small shift in mean value from that of the controls and did not improve the detection rate for Down's syndrome when added to the AFP/hCG/age combination. Median Frß-hCG levels in Down's syndrome were slightly higher than intact hCG levels, but replacing hCG with Frß-hCG reduced the predicted detection rate for Down's syndrome to 51% at a 5% false positive rate, due to the increased spread of values in the distributions of Frß-hCG, with this assay method, in unaffected and Down's syndrome pregnancies.

The third phase of this study was a test of the performance of the hCG/AFP/age combination in clinical practice. A prospective trial was carried out between July 1989 and June 1990, assaying hCG in addition to AFP in all serum samples routinely received from three Glasgow maternity hospitals. The hCG results for each pregnancy

were stored on a database and only analysed after delivery. A total of 7,830 pregnancies was tested and, using a threshold risk of 1:220, 53% of the Down's pregnancies were assigned to the high risk group and 6.2% of all pregnancies classified as 'screen positive'. This confirmed that enhanced detection of Down's syndrome pregnancies could be achieved in routine practice using hCG and AFP in combination with maternal age. AFP and hCG levels were shown to vary with maternal weight, with lighter women having increased levels and reduced levels being found in heavier women. Cases of threatened abortion, which are known in some cases to have elevated AFP levels, had unchanged levels of hCG. Levels of AFP in 81 twin pregnancies were approximately double those found in singleton pregnancies, with a median AFP level of 1.91 MOM and a median hCG level of 1.85 MOM. In 56 pregnancies affected by insulin dependent diabetes, the median value of both AFP and hCG was slightly reduced, at 0.94 MOM and 0.90 MOM respectively.

On the basis of this study hCG estimation was introduced in the west of Scotland prenatal screening programme in September 1991. Analysis of the first 30,084 pregnancies screened has shown that, using a threshold risk of 1:220, 1,523 women (5.1%) have been classified as 'screen-positive' and 26 Down's syndrome pregnancies identified in this high risk group. There were 37 Down's syndrome pregnancies in the whole screened population, giving a detection rate of 70%. The uptake of diagnostic testing by women in the high risk group has also increased, with 70% of women, regardless of age, opting for testing. Overall 21 of the 37 Down's syndrome pregnancies (56%) were prenatally diagnosed. In addition 2 out of 7 (29%) trisomy 18 pregnancies were identified in a second high

risk group of 87 women (0.3%) defined on the basis of low hCG.

Introduction of biochemical screening has lead to a fourfold improvement in the prenatal diagnosis rate for Down's syndrome in the whole west of Scotland pregnant population from 13% in 1986 to 48% in the year September 1991 - September 1992. Prenatal screening for chromosome abnormalities by biochemical methods in the second trimester is therefore more effective in practice than using maternal age alone as the criterion for selecting women for diagnostic testing.

SECTION 1

INTRODUCTION

1.1 PRENATAL SCREENING

Prenatal screening is a way of selecting from the pregnant population a group of women who are at the highest risk of having a child with a particular abnormality. A positive screening result does not indicate with certainty the presence of an abnormality, but classifies an expectant mother as having a risk of an abnormal fetus high enough to justify the use of diagnostic procedures to definitely confirm or exclude the presence of an abnormality. Negative screening results reduce, but do not exclude, the risk of an abnormality.

Various parameters define the effectiveness of a screening test (Cuckle and Wald, 1984, Connor, 1989). These are sensitivity, specificity, positive predictive value and negative predictive value. The sensitivity, or detection rate, is the correctly predicted proportion of the actual total who are affected. The specificity is the correctly predicted proportion of the actual total who are unaffected (The false positive rate is 1 - specificity). The positive predicted value is the proportion with a positive test result who are affected and the negative predicted value is the proportion with a negative test result who are unaffected.

For screening to be appropriate it must be for a clearly defined disorder of known prevalence. The screening test must be easily and reliably carried out on a sample that can be obtained without risk to the patient, such as maternal venous blood. There must be an advantage to early diagnosis and the test must have good

sensitivity and specificity. Screening should be both cost and benefit effective, both in a financial sense and also in relation to the benefits and disadvantages for the patients being offered screening. The performance of the screening test should be monitored by follow-up studies to see that it is effective.

Public opinion in both the UK and the USA would appear to favour screening during pregnancy (King's Fund Forum Consensus Statement, 1987, Faden *et al.*, 1987). The King's Fund Forum concluded that screening should be seen as a means of acquiring information that increases the scope of choice by the participants. However screening is only one possible approach to reducing disability. Primary prevention of environmentally determined conditions and improving the facilities and attitudes of society to physically and mentally impaired people must be part of a comprehensive approach. Participation in a screening programme should be an informed and considered decision and a woman's access to screening or diagnostic testing should be independent of any decision she may make about the continuation of the pregnancy.

1.1.1 SCREENING FOR NEURAL TUBE DEFECTS

The best example of a screening programme for fetal abnormality is the measurement of maternal serum alphafetoprotein (AFP) for the detection of neural tube defects. By 1974 it had been demonstrated that pregnancies affected by an open neural tube defect had elevated levels of maternal serum AFP (Brock *et al.*, 1973, Leek *et al.*, 1973, Brock *et al.*, 1974, Wald *et al.*, 1974). The UK

Collaborative Study on Alphafetoprotein in Relation to Neural Tube Defects (1977) determined that 84% of pregnancies where the fetus had an open neural tube defect were associated with maternal serum AFP levels ≥2.5 multiples of the median (MOM), and that 3% of unaffected pregnancies also had AFP levels ≥2.5 MOM. Maternal venous blood samples should be collected between 16-20 weeks gestation, the AFP level measured and the 3% of women with the highest AFP results selected for diagnostic testing, either amniocentesis to measure amniotic fluid AFP levels and test for specific acetyl cholinesterase (Brock, 1983), or detailed ultrasonography (Morrow *et al.*, 1991).

Maternal serum alphafetoprotein (AFP) screening in the second trimester of pregnancy has contributed to a substantial decline in the birth incidence of neural tube defects in the west of Scotland, since it was introduced in 1976 (Ferguson-Smith, 1983) and in other parts of the UK (Cuckle and Wald, 1987).

1.2 CHROMOSOME ABNORMALITIES

The report by Hook and Hamerton (1977), who combined data from six published studies from a total of 56,952 babies, showed that chromosome abnormalities occur in newborns at a rate of 6/1000. Of these 2/1000 are sex chromosome abnormalities, 2/1000 are balanced chromosome rearrangements and the other 2/1000 involve unbalanced rearrangements of the autosomes. Of these unbalanced rearrangements, 1.4/1000 have excess chromosome material in the form of an additional autosome (autosomal trisomies) and the

remaining 0.6/1000 are unbalanced structural rearrangements of the autosomes.

The most common autosomal abnormality is Down's syndrome (trisomy 21) which has a birth frequency of 1:800 (Hook and Hamerton, 1977). The other autosomal trisomies which are relatively common are Edwards' syndrome (trisomy 18) which has a birth frequency of 1:8000 (Hook and Hamerton, 1977) and Patau's syndrome (trisomy 13) which has a birth frequency of 1:20000 (Hook and Hamerton, 1977).

Of the autosomal trisomies, Down's syndrome is the most important both from the prenatal diagnosis point of view and also with regard to the health care and social needs of affected individuals. This is due to the greater frequency of Down's syndrome births and the increased life expectancy of Down's syndrome patients, which now has a median of 56 years (Dupont *et al.*, 1986). The median survival time for trisomy 18 and trisomy 13 in an unselected population has been shown to be 6.0 days and 2.5 days respectively (Goldstein and Nielsen, 1988). However around 20% of affected individuals survive longer than one month, and since the majority are hospitalised for most of their survival period, these other autosomal trisomies should also be considered during prenatal screening and diagnosis.

It has been demonstrated by Hook (1978), in a study of 101 pregnancies in which a fetal chromosome abnormality was identified prenatally by amniocentesis but elective abortion did not occur, that the rate of spontaneous fetal death is six fold higher in pregnancies with a chromosome abnormality than in those with a

normal karyotype. The rates of chromosome abnormalities found if prenatal diagnosis is carried out at mid-trimester will therefore be higher than those seen at birth. Cuckle *et al.* (1987) have estimated that the risks of a Down's syndrome fetus are 25% higher at mid-trimester than at birth. This was done by comparing the age-specific risk of Down's syndrome in the second trimester of pregnancy, derived from the European collaborative study of 52,965 amniocenteses in women aged 35-44 (Ferguson-Smith and Yates, 1984) with the corresponding age-specific risk at birth.

Hook *et al.* (1988), in a study of 4,481 chorionic villus samples (CVS), estimated that 21% of Down's syndrome pregnancies diagnosed at CVS at around 10 weeks gestation would be lost before the time when amniocentesis would be carried out at around 16 weeks gestation i.e. that the rate of Down's syndrome at 10 weeks is approximately 25% higher than at 16 weeks. Thus only around 63% of Down's syndrome fetuses present at 10 weeks gestation will therefore survive to term.

1.3 MATERNAL AGE RISKS

1.3.1 DOWN'S SYNDROME

The link between advancing maternal age and increasing risk of having a Down's syndrome child was first demonstrated by Penrose in 1933, who also showed that paternal age was an insignificant factor. With the development of chromosome banding techniques it was estimated by Juberg and Mowrey (1983), in a review of 30

previously published studies, that maternal origin accounted for 80% and paternal origin 20% of cases. However more recent investigations, using DNA polymorphisms, have shown that in only about 5% of cases was the extra chromosome 21 of paternal origin (Sherman *et al.*, 1991, Antonarakis *et al.*, 1991). This much lower rate of extra chromosomes of paternal origin provides further evidence that paternal age has little or no effect on the rate of Down's syndrome.

There have been eight large published surveys of Down's syndrome in live births which specify the risk for single age years (Hook and Chalmers, 1977, Hook and Fabia, 1978, Hook and Lindsjö, 1978, Trimble and Baird, 1978, Sutherland *et al.*, 1979, Young *et al.*, 1980, Koulischer and Gillerot, 1980, Huether *et al.*, 1981). Hook and Chalmers (1977) ascertained 933 Down's syndrome cases from 1,729,909 live births from birth certificates between 1963 and 1974 in New York State. To allow for under-ascertainment the observed number of cases was multiplied by 2.66 to estimate the true age-specific risk. This factor was derived from a sub-set of 301 cases of Down's syndrome identified from cytogenetic records. 113 of these had Down's syndrome mentioned on the birth certificate.

Hook and Fabia (1978) identified 1,250 Down's syndrome cases in 832,531 live births in the white population between 1958 and 1965 in Massachusetts. The cases of Down's syndrome were ascertained from surveys of hospitals and institutions and from cytogenetic records. Unaffected maternal age data was only available in five year intervals, and rates from individual maternal age years were

estimated by comparison with the rates in New York.

A study in Sweden by Hook and Lindsjö (1979) analysed 438 Down's syndrome births in 330,859 live births between 1968 and 1970. The Down's syndrome cases were identified from a variety of community sources and population demographic information on single-year maternal live births was available.

Trimble and Baird (1978), using British Columbia data from 1961 to 1970, ascertained 519 Down's syndrome births out of a total of 354,880 live births. The data were obtained by linking records of children with Down's syndrome at the British Columbia Health Surveillance Registry (BCHSR) to the appropriate birth registrations to derive maternal ages. The BCHSR uses numerous sources of ascertainment and completeness of reporting of Down's syndrome cases is thought to be very high. Single year maternal age rates of live birth were available.

In a survey of Down's syndrome in South Australia between 1960 and 1977 Sutherland *et al.* (1979) identified 447 Down's syndrome births. There were 375,488 live births during this period with single-year maternal ages available. Ascertainment of Down's syndrome cases was from the records of public institutions.

Seventy cases of Down's syndrome were identified in South Wales between 1968 and 1976 by Young *et al.* (1980). Cases were ascertained from the Cardiff Birth Survey and from cytogenetic records. The number of live births during the study period was 46,048. Maternal age in the general population was recorded only

in five-year intervals and single-year rates were derived by comparison with the Swedish study (Hook and Lindsjö, 1978).

The report by Koulischer and Gillerot (1980) concerns a group of 268 Down's syndrome patients born in South Belgium between 1971 and 1978. The total number of live births during this time was 102,863. Down's syndrome cases were identified by cytogenetic examination of all newborns thought to be affected on examination by an obstetrician and a paediatrician. A single-year age distribution was available.

The study by Huether *et al.* (1981) was carried out on births in the white population between 1970 and 1979 in Ohio. It was conducted in a similar way to that of Hook and Chalmers (1977) by comparing data on Down's syndrome with that available from cytogenetics laboratories and using this information to estimate the level of under-reporting on birth certificates. Single-year maternal age rates were available. 851 Down's syndrome births were identified from birth certificates and the true level of Down's syndrome was estimated to be 2.74 times this. There were 1,460,449 live births during this time. A regression equation for Down's syndrome risk was calculated using the model of Lamson and Hook (1980) and the regression equation corrected for under-ascertainment to derive risks for individual maternal ages.

Cuckle *et al.* (1987) compiled risks from these eight surveys of Down's syndrome in live births, and applied the constant-plus-exponential (CPE) model proposed by Lamson and Hook (1980) to derive a regression equation for maternal age risk. The

equation is in the form

$$y = a + \exp(b + cx)$$

where y is the rate in live births, x is maternal age and a, b and c are constants. Lamson and Hook (1980) proposed that this model is consistent with a continuously accumulating biological process resulting in Down's syndrome, in which the rate of increase is proportional to the level already reached, analogous to the type of curve produced by an infectious process.

A weighted average of the separate risk estimates, on a log scale, from each of these studies was produced. To reduce random error a regression analysis was performed. The regression equation was

$$p = 0.000627 + e^{(-16.2395 + 0.286MA)}$$

were MA is maternal age in years. The risk of having a Down's syndrome birth is

$$1:(1 - p)/p$$ (See Table 5-3)

Since these risks are derived from 4528 Down's syndrome pregnancies and over 5,000,000 live births they are the best estimates of risk of a Down's syndrome birth available.

All these data have been gathered in Caucasian populations. Several studies have been carried out to investigate whether there are any racial differences in Down's syndrome rates in other ethnic populations. The evidence for this is conflicting, with different studies reporting different results. In the black population the Down's syndrome rate has been shown to be higher (Hook and Harlap, 1979), unchanged (Marmol *et al.*, 1969) and lower (Stark and White, 1977) than in Caucasian populations. Similarly conflicting evidence has been shown for Asian populations, with

reports showing higher rates (Hook and Harlap, 1979), unchanged rates (Verma and Singh, 1975) and lower rates (Rogers, 1986) when compared with the white population. In the Japanese population Matsunaga (1967) found rates of Down's syndrome in different maternal age groups not significantly different from that found in Caucasian populations. In the Chinese population rates of Down's syndrome lower than those in the Caucasian population have been reported (Emanuel *et al.*, 1972). In the light of any definite evidence to the contrary a reasonable assumption at this stage is that Down's syndrome risks derived from the Caucasian population can be applied to other ethnic groups. Further studies, carefully controlled for maternal age, would be necessary to test whether there is any difference in Down's syndrome risks between different ethnic groups.

1.3.2 OTHER AUTOSOMAL TRISOMIES

The risk of having a pregnancy associated with trisomy 18 or trisomy 13 also increases with maternal age. In a review of the literature Taylor (1968) found a mean maternal age at birth of 31.7 years in 153 cases of Edwards' syndrome, and 31.6 years in 74 cases of Patau's syndrome, compared with around 25 years in the unaffected pregnancies. Hook *et al.* (1979) compared rates of Down's syndrome and trisomy 18 in single-year maternal ages in livebirths in the same population and found similar rates of change relative to maternal age, with a ratio of trisomy 18 to Down's syndrome of 0.135 (CI 0.052 - 0.297). From these data Hook (1981) produced estimated rates per thousand live births in single

year intervals from 15-49 years for Down's syndrome, trisomy 18 and trisomy 13. The data for trisomy 13 assumed the same rate of change with age as that found for Down's syndrome and trisomy 18. Ferguson-Smith and Yates (1984) reported on the European collaborative study of chromosome abnormalities in 52,965 amniocenteses carried out for maternal age 35 years and over. The rates found in this study show the same rate of change as those predicted by Hook (1981) but are higher due to the known fetal loss of autosomal trisomies between mid-trimester prenatal diagnosis and birth (Hook, 1978).

1.4 MATERNAL AGE IN SCREENING FOR CHROMOSOME ABNORMALITIES

The association between risk and maternal age has been the basis of a widely used method to screen pregnant women for chromosome abnormalities. Since the early 1970s it has been established practice to offer diagnostic amniocentesis, and more recently CVS, to women at or above a certain age, commonly 35-37 years. As a screening test maternal age meets the criteria of simplicity and acceptability to the patient as it depends on recognition of an age threshold rather than knowledge or interpretation of a specific risk figure. However it is an ineffective method of screening. For example, in the west of Scotland using age 35 as a cut-off, 6.7% of the pregnant population require diagnostic testing, but the sensitivity is only 30%, which represents the proportion of autosomal trisomy pregnancies within this age group (Figure 1-1). The positive predictive value of the test is also poor since only one abnormality will be found for every 125

diagnostic tests performed. This screening method has made little impact on the number of children born with Down's syndrome in the west of Scotland (Stone *et al.*, 1989) since the poor sensitivity of the test has been compounded by the low uptake of diagnostic testing (less than 40%) in women aged 35 years and over. However even if all eligible women of 35 years and over had had prenatal diagnosis this screening method would still fail to access the 70% of affected pregnancies which occur in women under 35 years.

1.5 BIOCHEMICAL MARKERS FOR CHROMOSOME ABNORMALITIES

1.5.1 ALPHAFETOPROTEIN

The association between low maternal serum alphafetoprotein (AFP) and fetal aneuploidy was first reported by Merkatz *et al.* (1984), who first observed levels 'below sensitivity' of the assay in two patients with trisomy 18. Following this with a study of maternal serum AFP in 52 chromosomally abnormal pregnancies, 21 out of 25 Down's syndrome (84%), 11 out of 12 trisomy 18 (92%), 3 out of 3 trisomy 13 and 7 out of 12 (58%) sex chromosome abnormalities were found to have AFP values below the median of unaffected pregnancies. This result was confirmed for Down's syndrome by Cuckle *et al.* (1984), Seller (1984), Guibaud *et al.* (1984), Tabor *et al.* (1984), Trigg *et al.* (1984), Fuhrmann *et al.* (1984) and Voigtländer and Vogel (1985), who reported levels with median values ranging from 0.72 - 0.80 MOM for Down's syndrome cases, with 80 - 88% of values below the median of control cases. However

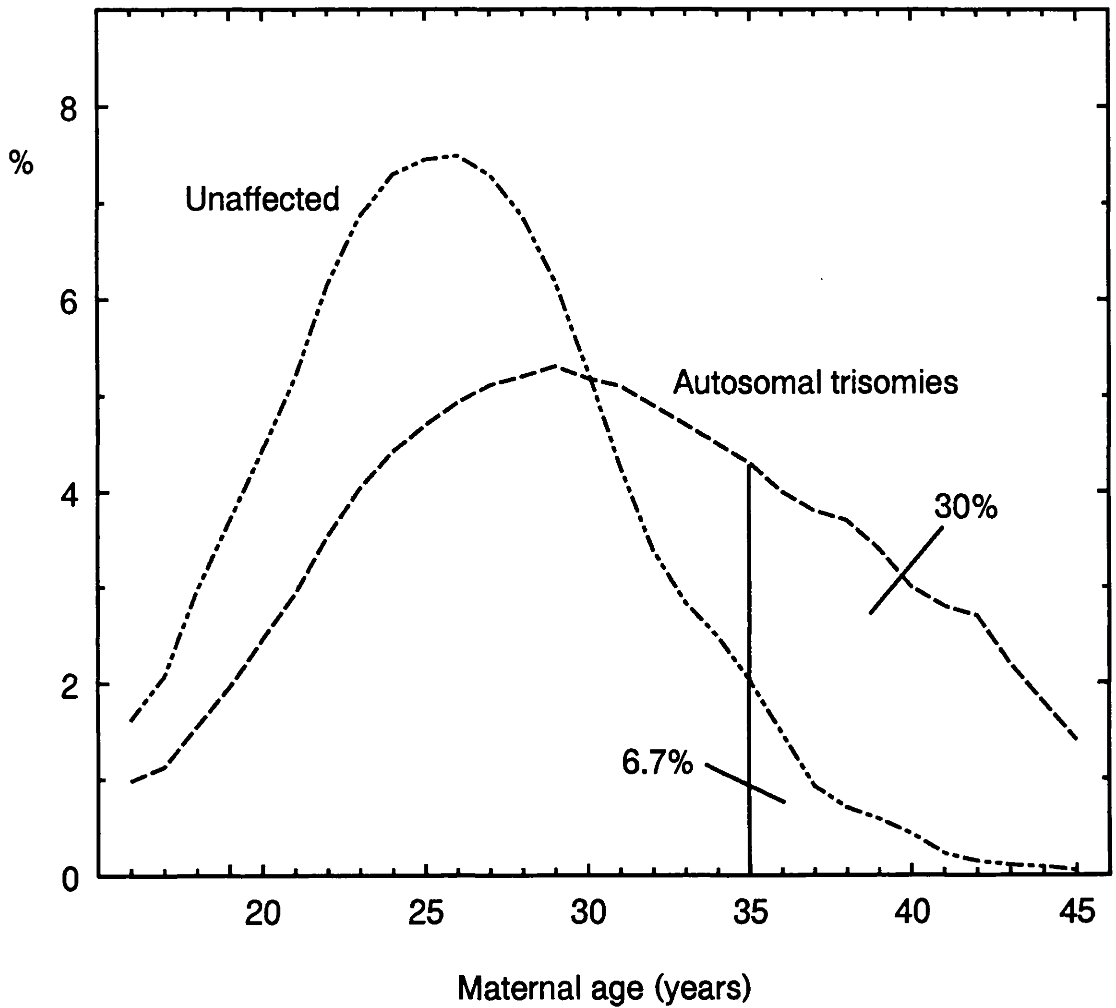


Figure 1 -1

Maternal age distributions for autosomal trisomy and unaffected pregnancies in the west of Scotland

Hershey *et al.* (1985) found, in a series of 32 cases of autosomal trisomy, (28 Down's syndrome and 4 Trisomy 18) only slightly lowered maternal serum AFP levels (0.87 MOM) and Cowchock and Ruch (1984) found no difference between Down's syndrome and control cases in a study of 40 cases of trisomy 21.

Cuckle *et al.* (1984) calculated a relative risk of Down's syndrome at each different level of AFP in MOM and proposed using a sliding scale of MOM as a cut-off for different maternal ages. Using this method a 40% detection rate for Down's syndrome was predicted, at a 6.8% false positive rate.

Baumgarten (1985) proposed an algorithm for calculating a woman's Down's syndrome risk based on her age and AFP level, and reported a prospective trial of this type of screening in women aged under 35 years (Baumgarten *et al.*, 1985). The algorithm was based on a Gaussian rather than log Gaussian distribution for AFP levels in MOM, However, in practice, screening 9059 women aged less than 35 years, 444 (4.9%) had a risk greater than 1:250. Of these, 6 women had a Down's pregnancy and one a trisomy 18 pregnancy, giving overall odds of finding an affected pregnancy of 1:63. The number of autosomal pregnancies in women in the low risk group was not reported.

Spencer and Carpenter (1985) reviewed a four year period when 27,064 pregnancies were screened for neural tube defects using AFP. Twenty seven Down's syndrome cases were identified in the screened population and these had a median value of 0.82 MOM of unaffected pregnancies. Using the strategy suggested by Cuckle *et*

al. (1984) in this population the detection rate was only 14.8% and the false positive rate 8.6%, and the authors suggested that the fetal loss rate would be higher than the detection rate and therefore suggesting caution in the introduction of this type of screening.

Murday and Slack (1985) reported on a 12 month retrospective study of maternal age/AFP screening in the North East Thames region of London. There were 78 Down's syndrome pregnancies identified and AFP measurements were available for 45 of these. Fourteen of the Down's syndrome pregnancies were prenatally diagnosed. The affected pregnancies had reduced levels of maternal serum AFP at 0.63 MOM of unaffected pregnancies. The authors proposed a method of risk calculation from the overlapping log Gaussian distributions, based on the Bayes calculation method of Dennis and Carter (1978). They estimated that by offering amniocentesis to women aged 32 or greater who had a risk based on age and AFP level of 1:200 or greater, an extra 12 Down's syndrome cases would have been detected, if all of the women in the high risk group had had a diagnostic test. The authors suggested that their better detection rate, compared with that of Spencer and Carpenter (1985) may in part be attributed to more older mothers in their population. The lower levels of AFP in the Down's cases (Median of 0.63 MOM versus 0.83 MOM) may also have been a contributory factor.

Another retrospective analysis of results from a screening programme in Canada was reported by Doran *et al.* (1986), who considered results not only from mothers with Down's syndrome

pregnancies but also with trisomies 18 and 13. They reviewed 6851 maternal serum AFP results from samples taken prior to amniocentesis, and a further 6505 results from women having AFP screening. There were 61 autosomal trisomy pregnancies, consisting of 46 Down's syndrome, 10 trisomy 18 and 5 trisomy 13. The levels in the Down's and trisomy 18 pregnancies were reduced, at 0.79 MOM and 0.64 MOM respectively. The levels in the trisomy 13 pregnancies was not significantly different from the controls, at 1.19 MOM. Reviewing their results the authors suggested that offering amniocentesis to women under 35 who had AFP levels of less than 0.5 MOM would detect 20% of fetal autosomal trisomies and put 5% of women in the high risk group, with overall odds of finding an affected pregnancy of 1:180.

Martin and Liu (1986) produced risk tables for both birth and mid-trimester which included prior risk, risk if AFP ≤0.4 MOM and risk if AFP >0.4 MOM from the results of two other published studies. Hershey *et al.* (1986) also published risk tables based on results from 28 Down's cases from their own centre, combined with 137 other previously published results from other centres. These risks were based on maternal age and a sliding scale of AFP values. Risks were calculated by Bayes theorem.

Results of a prospective trial of age/AFP screening in 51,141 women under 35 years from eight centres were reported by Palomaki (1986), on behalf of the New England Collaborative Down Syndrome Prenatal Screening Study. Of the 1050 women (2.1%) assigned to the high risk group (2.1%), 807 (77%) opted for amniocentesis. Amongst these women 12 autosomal trisomy pregnancies (8 Down's syndrome

and 4 trisomy 18) were identified, giving overall odds of an affected pregnancy of 1:58. No birth outcome information on the women who did not have a diagnostic test, from either the high or low risk group was available at the time of publication. The study concluded that this method of screening appeared feasible in practice.

Ashwood *et al.* (1987) carried out a retrospective study on maternal serum samples taken for AFP analysis prior to amniocentesis from 3,411 women. There were 71 fetal chromosome abnormalities in these patients, including 26 Down's syndrome and 10 trisomy 18. Risks of Down's syndrome were calculated from the cumulative proportions of unaffected and Down's syndrome samples and compiled Down's syndrome maternal age risks produced by Hook (1981) after adjusting these to mid-trimester. The calculation of risk was by Bayes theorem. Due to the relatively small numbers of Down's syndrome samples it was not possible to derive a smooth cumulative proportion curve and the assumption was made that the affected distribution was shifted 0.3 MOM from the control distribution and that any point on the Down's distribution was equivalent to the same point on the curve - 0.3. Since AFP fits a log Gaussian rather than a Gaussian distribution (Cuckle *et al.*, 1987) this is not a valid assumption.

Palomaki and Haddow (1987) were the first to suggest the likelihood ratio method of calculating Down's syndrome risks. This method derives risks from the overlapping log Gaussian distributions of control and Down's syndrome samples. The likelihood ratio at any given AFP result in MOM is the distance

from a point on the base line to the curve representing the affected population divided by the distance to the unaffected curve. The height to a Gaussian distribution can be calculated by a formula which uses the mean and standard deviation of the distribution (Cuckle and Wald, 1984).

Cuckle *et al.* (1987) extended this method of risk calculation suggested by Palomaki and Haddow to include maternal age risks from a compilation of eight other studies (see Section 1.3). These were combined with risks derived from AFP results from their own series of 68 Down's syndrome samples and 36,645 unaffected pregnancies. They described how to calculate a likelihood ratio for any given AFP value in MOM using a formula which utilizes the means and standard deviations of the Down's syndrome and unaffected distributions. The authors compiled risk tables giving an individual woman's risk of having a Down's birth, based on her age and AFP level, for gestations based on last menstrual period or ultrasound, and for AFP values which included or excluded a correction for maternal weight. This study also derived false positive rates and detection rates for screening policies using differing risk thresholds as a cut-off, predicting, for example, a 36% detection rate for Down's syndrome at a 5.4% false positive rate using a 1:300 risk threshold. It concluded that screening using AFP/age was more efficient than using maternal age alone and that where prenatal screening for neural tube defects was in progress it was also justifiable to use the results for screening for Down's syndrome.

Tabor *et al.* (1987), using data derived from 86 Down's syndrome

pregnancies and 2018 unaffected singleton controls, used Bayes theorem to derive risks from the log Gaussian distributions of affected and unaffected pregnancies. From these they constructed an iso-risk curve equal to a risk of 1:400 to select women for amniocentesis. Women whose AFP/age combination fell below the iso-risk line were in the high risk group and would be offered amniocentesis. Using their data, where the Down's cases had a median of 0.64 MOM, they predicted, in their population, a detection rate of 53% for Down's syndrome with a false positive rate of 9.4%. This compares with a detection rate of 28% at a false positive rate of 6.9% using maternal age ≥35 years in the same population.

Lindenbaum *et al.* (1987) reported on maternal serum levels of AFP in 50 cases of trisomy 18. Of these 38 (76%) had neither exomphalos nor neural tube defect and for these cases the median level of AFP was 0.60 MOM with 30 of the cases having values below the median. Screening programmes for Down's syndrome using low AFP levels will therefore also detect trisomy 18 pregnancies.

Di Maio *et al.* (1987) reported on prospective screening with AFP/age in 35,797 women of whom 34,354 (96%) were aged less than 35 years. Risks were calculated by the method of Baumgarten *et al.* (1985) which assumes a Gaussian rather than log Gaussian distribution for AFP. Using a risk threshold of 1:270 1814 (5.3%) were assigned to the high risk group. Of these 18% were found to have overestimated gestation. Of the 1451 with a confirmed risk of ≥1:270, 1102 (76%) elected to have amniocentesis. Of the women in the confirmed high risk group 9 had a fetus with Down's syndrome

and 8 of these were prenatally diagnosed. In addition 3 cases of trisomy 18 and one of trisomy 13 were assigned to the high risk group. Screening identified 9 out of 27 Down's cases (33%), 3 out of 6 trisomy 18 cases (50%) and 1 out of 3 trisomy 13 cases (33%).

Another prospective series, with incomplete ascertainment of Down's cases, was reported by Lustig *et al.* (1988). They reported on 174,784 women screened in California. 3,939 women (2.25%) were assigned to the high risk group (risk ≥1:365) on the basis of risks derived from their AFP level and maternal age, using the risk calculation method of Cuckle *et al.* (1987) and the Down's syndrome incidence figures of Hook and Chalmers (1977). 2,552 of these women remained in the high risk group after ultrasound assessment of their gestation and 1940 (76%) had amniocentesis. There were 23 Down's syndrome pregnancies amongst these women of which 17 were diagnosed by amniocentesis. Five of the remaining cases were missed due to an initial policy of requesting a repeat sample and assigning women to the low risk group if this second sample gave a risk less than the threshold. This repeat policy was changed in the latter half of the period reviewed on recognition of the issue of regression to the mean (Haddow *et al.*, 1986). Amongst the women in the high risk group there were also 4 cases of trisomy 18, 1 trisomy 13, 2 Turner's syndrome, 4 Turner mosaics, 1 Klinefelter's syndrome, 1 Klinefelter mosaic and 1 triploidy. No data were available on chromosome abnormalities in women assigned to the low risk group.

Hook (1988) discussed the validity of using AFP/age to reassure older women (age ≥35 years), who have relatively high AFP result

which gave a risk of Down's syndrome lower than that associated with their age, and who consequently might avoid the need for amniocentesis. The criticisms of this approach were (a) that Down's syndrome risks from AFP levels were derived from a mathematical model of a relatively small amount of data rather than from direct observation, (b) that the assumption is made that AFP distributions do not change with maternal age and (c) that the risks and relative proportions assigned to high and low risk groups at the same maternal age and AFP level vary between different studies (Ashwood *et al.*, 1987, Cuckle *et al.*, 1987, Di Maio *et al.*, 1987, Palomaki and Haddow, 1987, Tabor *et al.*, 1987)

Wald and Cuckle (1988) discussed this last point further and compared the methods used to derive risks in these five studies. Two were based on an inappropriate mathematical model (Ashwood *et al.*, 1987, Di Maio *et al.*, 1987). The risks of Tabor *et al.* (1987) are discrepant from the others due to a lower median level of AFP (0.64 MOM) found compared to that found by Cuckle *et al.* (1987) and Palomaki and Haddow (1987) (0.72 MOM) who used the same data set. The small differences in the risks of Cuckle *et al.* (1987) and Palomaki and Haddow (1987) can be attributed to the different method used to calculated the standard deviation. Palomaki and Haddow (1987) used the interquartile range whereas Cuckle *et al.* (1987) used the 10th - 90th centile range. This latter method is thought to be more appropriate.

The economics of using AFP/age screening have been investigated by Gill *et al.* (1987), Swint and Greenberg (1988) and Wald and Cuckle (1988). Gill *et al.* (1987) carried out a cost benefit analysis of

the costs of screening, diagnosis and termination of pregnancy compared with the lifetime costs of a Down's birth, taking into account that a termination of pregnancy will probably be replaced by an unaffected pregnancy. They concluded that the financial benefits of screening either by age alone or by AFP/age are favourable and that not offering AFP/age screening would be discriminating against younger women. Swint and Greenberg (1988) concluded that if screening by maternal age is economically justifiable then offering amniocentesis to younger women on the basis of AFP/age is also economically viable. Wald and Cuckle (1988) pointed out that since the overall odds of finding an affected pregnancy are greater in women selected by AFP/age rather than by age alone the net cost of detecting a Down's pregnancy is lower when screening by AFP/age.

1.5.2 UNCONJUGATED ESTRIOL

Greater sensitivity is potentially possible using additional information from the analysis of other pregnancy markers in maternal serum. It had been observed by Jorgensen and Trolle (1972) that total urinary estriol secretion in the third trimester of pregnancy was lower in Down's syndrome pregnancies than in unaffected pregnancies. Canick *et al.* (1988) and Wald *et al.* (1988a) reported lower second trimester levels of unconjugated estriol (UE3) in Down's syndrome pregnancies. Canick *et al.*, in 22 Down's syndrome pregnancies and 110 matched controls found a median level of 0.79 MOM in the Down's pregnancies. Unconjugated estriol, unlike total estriol, is almost entirely derived from the

fetus and placenta (Siiteri and MacDonald, 1966).

Wald et al. found levels of 0.73 MOM in 77 Down's syndrome pregnancies, compared with the levels in 385 controls. Significant correlation was found between AFP and UE3. Extending the approach to risk calculation previously described for AFP and age (Palomaki and Haddow, 1987, Cuckle *at al.*, 1987) to include UE3, and allowing for the correlation between AFP and UE3, a detection rate for Down's syndrome of 45% was predicted with a false positive rate of 5.2%, using a risk threshold of 1:250.

1.5.3 HUMAN CHORIONIC GONADOTROPHIN

Bogart *et al.* (1987) found elevated levels of total human chorionic gonadotrophin (hCG) and free α-subunit hCG in 17 cases of Down's syndrome compared with the levels in 74 controls. Samples were taken between 18 and 25 weeks after fetal chromosome analysis had been carried out following amniocentesis. Screening using elevated levels of hCG would be more effective than using low levels of AFP.

Wald *et al.* (1988b), in a study of hCG levels in 77 Down's syndrome pregnancies and 385 controls, found a median hCG level in the Down's syndrome pregnancies of 2.04 MOM and predicted a 55% detection rate by combining hCG and AFP results with maternal age risks, as described for UE3. By also including UE3 results the predicted detection rate was increased to 61% for a 5% follow up rate. This combination of analytes (AFP/hCG/UE3) has become known

as 'The Triple Test'.

Arab *et al.* (1988) suggested an alternative approach for the use of hCG in screening for Down's syndrome by combining hCG and AFP results as a ratio, and from a study of 29 Down's syndrome pregnancies predicted a 60% detection rate for a 10% amniocentesis rate, but the data were not combined with maternal age risks.

1.5.4 PREGNANCY-SPECIFIC β_1-GLYCOPROTEIN

Bartels and Lindemann (1988) found elevated levels of maternal serum pregnancy-specific β_1-glycoprotein (SP_1), similar in magnitude to that reported for hCG. In 24 pregnancies with Down's syndrome a median level of SP_1 of 2.1 MOM was found compared with the levels in 34 unaffected controls, with 18 out of 24 values being above the 90th centile. Samples were taken between 16 and 19 weeks of gestation after patients had undergone amniocentesis and fetal chromosome analysis carried out.

1.6 DEVELOPING SCREENING

At the commencement of this project in 1988 the possibility therefore existed to increase the detection rate for Down's syndrome in the second trimester of pregnancy to around 60% using a combination of maternal serum markers and maternal age risks. However the effect of this type of screening on the detection of other types of chromosome abnormalities, especially other

autosomal trisomies, was not known and there were many unanswered questions regarding the efficacy of multimarker screening in routine practice.

SECTION 2

AIMS

The study can be divided into three main parts:

2.1 PHASE 1

Retrospective data analysis of maternal serum AFP results in 142 autosomal trisomy pregnancies to establish the parameters of the distributions in abnormal and unaffected pregnancies and derive risk tables applicable to routine AFP screening.

2.2 PHASE 2

Retrospective analysis of four other pregnancy markers (alphafetoprotein, human chorionic gonadotrophin, unconjugated estriol and the free ß subunit of hCG) in stored maternal serum samples from pregnancies with Down's syndrome and other chromosome abnormalities to establish the variation in levels between unaffected and abnormal pregnancies, and to look at ways that these variations might be exploited to provide risk estimation for population screening

2.3 PHASE 3

To establish the impact of biochemical screening for chromosome abnormalities in a large unselected population, by determining detection rates, false positive rates and the impact on prenatal diagnosis rates and birth incidence.

SECTION 3

MATERIALS

3.1 PATIENT SAMPLES

The Duncan Guthrie Institute of Medical Genetics, Yorkhill, Glasgow provides a comprehensive Medical Genetics Service to 3.0 million people in the west of Scotland. One aspect of this service is prenatal screening for women in 8 Area Health boards (Argyll and Clyde, Ayrshire and Arran, Dumfries and Galloway, Forth Valley, Greater Glasgow, Highland, Lanarkshire and Western Isles). Screening for neural tube defects started in 1976 and was extended in 1987 to include chromosome disorders, mainly Down's syndrome. In this region there are around 37,500 births per year and 80% of pregnant women (30,000 per year) choose to have a prenatal screening test.

The majority of venous blood samples (5-10 mls) are collected at 15-20 weeks gestation from women attending one of the 18 maternity units in the west of Scotland. A few samples are taken directly by GPs. Samples are sent to the Biochemical Genetics Division of the Institute of Medical Genetics where they are given a laboratory accession number, separated, an aliquot of serum used for assay and the remainder (1-3 mls) stored at -20°C. A standard request form accompanies the sample, giving details of the patient and her pregnancy: Name, date of birth, weight, gestation (derived from menstrual dates, by clinical examination and ultrasound estimation, usually derived either from bi-parietal diameter or crown-rump length), history of previous neural tube defect or chromosome abnormalities, and details of pregnancy complications (threatened abortion, twins, insulin dependant diabetes, anticonvulsant therapy, invasive diagnostic procedures). Patient

and sample details are entered into a computerised database which is used to generate assay worklists. Following analysis of samples, results are merged with patient information, reports are generated and completed records archived as printout or on disc.

The Medical Genetics department also provides a cytogenetic service for all of the previously above Health Boards apart from Highland region which has its own cytogenetics laboratory. Chromosomally abnormal pregnancies, identified either pre- or post-natally, can be ascertained from inspection of departmental cytogenetic records. Information on patients from Highland region was obtained from the Cytogenetics Department in Raigmore Hospital, Inverness.

3.1.1 RETROSPECTIVE AFP STUDY

Pregnancies affected by Down's syndrome or other autosomal trisomies, diagnosed either at birth or prenatally between April 1982 and May 1987 in the west of Scotland, were identified from departmental cytogenetic records. After exclusion of twin pregnancies and cases complicated by ventral wall or neural tube defects or other structural abnormalities, maternal serum AFP results were available from the screening archive for 142 affected pregnancies screened between 16 and 20 weeks gestation (32 terminations and 110 live births). The study group consisted of 114 Down's syndrome, 19 with trisomy 18 and 9 with trisomy 13. Maternal serum AFP results at 16-20 weeks gestation from 113,045 unaffected pregnancies (without autosomal trisomy or neural tube

defect) tested during the same period were used for comparison as a control group. If more than one AFP result was obtained in any pregnancy, only the first was used.

Estimates of gestation were based mainly on the time since the first day of the last menstrual period (LMP), modified by ultrasound as suggested by Rossavik and Fishburn (1989). Ultrasound measurement was used only were the date of the LMP was not available or were there was a discrepancy of ±2 weeks in the estimated gestation by ultrasound. All gestations were established by this method using the information obtained at the time of sampling. This therefore reflects the quality of information likely to be available in routine screening programmes and avoids dependence on a single dating method which was not available for all samples.

3.1.2 RETROSPECTIVE STUDIES ON OTHER PREGNANCY MARKERS

In addition to AFP, retrospective analysis using stored samples was undertaken for four other markers: hCG, SP1, UE3 and the free ß subunit of hCG. Sixty-five thousand serum samples collected prospectively in the west of Scotland at 15-20 weeks gestation for routine AFP estimation between January 1987 and March 1989 provided the basic source material for these studies. After routine AFP estimation, sera were stored at -20°C until the outcome of the pregnancy was known. An initial pool of 78 chromosomally abnormal pregnancies were identified from which stored serum was available. The chromosomally abnormal cases are

listed in Table 3-1. In any abnormal case where two consecutive samples were available only the first sample was used. Five control sera were selected for each case, matched for maternal age (within 12 months), completed weeks of gestation and time in frozen storage (within 6 months). All control specimens were selected without reference to their AFP level. Additional cases of Down's syndrome and trisomy 18 were added to the study group as they were identified within the samples stored following routine screening. In all, a panel of 120 serum samples from chromosomally abnormal pregnancies were accumulated for use in the retrospective analyses of pregnancy markers (81 Down's syndrome, 12 trisomy 18, 4 trisomy 13, 10 unbalanced translocations, 4 balanced translocations and 9 sex chromosome abnormalities). To avoid repeated thawing and freezing, samples were recovered from storage, thawed once, divided into small aliquots and refrozen pending further analyses.

Gestational age (completed weeks) for both affected and unaffected pregnancies was based mainly on the time since the first day of the last menstrual period (LMP i.e. by dates). Ultrasound estimates of gestation were used only for those patients in whom either the LMP was not available or there was a discrepancy of at least ±2 weeks in gestational age calculated using dates and ultrasound.

3.1.3 PROSPECTIVE hCG TRIAL

Between July 1989 and June 1990 all samples sent for routine AFP

Table 3 - 1

Chromosomally abnormal pregnancies used in retrospective studies of pregnancy markers.

Abnormality	n		Karyotype
Down's syndrome	49		
Trisomy 18	4		
Trisomy 13	4		
Unbalanced Translocations	8		46,XY 2p+
			46,XY,del(21)(q22qter)
			46,Y,-X,+der(X)(t;11)(p22p15)mat
			46,XX,-11,+der(11)t(4;11) (q31.1q25) pat
			46,XY,del(4)(q12q21.3)
			46,XX,del(11)(q24qter)
			45,XY,-10,-14,+t(10;14)(p13q11.2)
			46,XY/47,XY+m
Balanced Translocations	4		46,XY,inv(2)(p11q13)
			46,XX,t(9;10)(q32p13)pat
			45,XX,t(13q;15q)
			46,XX,t(12;16)(q13p11.2)
Sex Chromosome Abnormalities	9	3	45,X
			49,XXXXX
			47,XXY
		3	47,XYY
			45,X/46,XY

estimation from three Glasgow maternity hospitals (Glasgow Royal Maternity Hospital, The Queen Mothers Hospital and Rutherglen Maternity Hospital) also had hCG estimation carried out. The hCG results were stored and only analysed after delivery. 7,830 pregnancies were tested and 16 Down's syndrome and 2 trisomy 18 pregnancies identified amongst the women screened.

3.1.4 CLINICAL PRACTICE

In July 1987,The west of Scotland maternal serum screening service for neural tube defects was adapted to include the reporting of risks of an autosomal trisomy in individual pregnancies, using the risks derived from maternal age and AFP result. From September 1991 hCG estimation, in addition to AFP, was carried out routinely on all samples received by to the west of Scotland prenatal screening service. Table 3-2 gives a summary of the number of women screened and uptake of screening from July 1987 to September 1992.

Table 3 - 2

Births, pregnancies screened and uptake of screening in the west of Scotland.

Time	No.births	No.women screened	Uptake of screening	Gestation range (weeks)
July 87- Dec 90	128,824	100,481	78%	16-20
Sept 91- Sept 92	37,500*	30,084	80%	15-20

* Estimate

SECTION 4

METHODS

4.1 ASSAY METHODS

4.1.1 IMMUNOASSAYS

Immunoassays utilize the specific binding between an antibody and an antigentic substance to quantify the levels of the antigen in biological fluids. A prepared antibody may be polyclonal i.e. containing many different antibodies with differing specificities to multiple epitopes (binding sites) on the antigen molecule, or monoclonal i.e. homogeneous in specificity and recognising only one epitope on the antigen. Polyclonal antibodies are the conventional serum product of an immunised animal. Monoclonal antibodies are prepared from a cell line derived from a single antibody-producing cell immortalised by fusion to a B lymphocyte tumour cell line to form a 'hybridoma' clone.

Three types of immunoassay were used in this project, radioimmunoassay (RIA), immunoradiometric assay (IRMA) and rocket immunoelectrophoresis (IEP).

4.1.1.1 RADIOIMMUNOASSAY

In this technique labelled antigen competes with test antigen to complex with limited antibody in liquid phase. Either polyclonal or monoclonal antibody can be used but the antibody must have a high affinity for the antigen and also stringent specificity. The antibody-antigen complex is precipitated and the radioactivity in the bound (precipitated) fraction counted. The radioactive counts

obtained are inversely proportional to the amount of antigen in the sample (Catty and Murphy, 1989).

4.1.1.2 IMMUNORADIOMETRIC ASSAY

This technique uses radiolabelled monoclonal antibody to measure the amount of antigen in a sample. In a 'sandwich' (two-site) assay, solid phase antibody (polyclonal or monoclonal antibody bound to beads or plastic tubes) binds antigen and the presence of bound antigen is detected by the use of a second radiolabelled antibody (monoclonal). Antibody is present in excess. Free and bound labelled antibody are separated by one or more wash steps. The radioactivity in the bound fraction is counted. The counts obtained are proportional to the amount of antigen present in the sample (Catty and Murphy, 1989).

4.1.1.3 ROCKET IMMUNOELECTROPHORESIS

This method combines the electrophoretic migration of antigens in agarose with immunoprecipitation in the gel. Test samples are loaded into wells in an agarose plate containing antibody. Electric current is applied to move the antigen into the gel. Gels are prepared and run at a pH at or close to the isoelectric point of the antibody to prevent its migration from the gel. Sharply pointed precipitation peaks ('rockets') form as the antigen accumulates bound antibody during migration. The area of the rockets is proportional to the antigen concentration (Catty and Raykundalia, 1988).

4.1.2 ALPHAFETOPROTEIN

AFP was first identified in 1956 (Bergstand and Czar, 1956), but its function is still unknown. It is a glycoprotein, with a molecular weight of 69,000. Different molecular forms of AFP have been identified, which have differences in the amount and structure of carbohydrate residues of the molecule. AFP is synthesised by the yolk-sac and the fetal liver. The yolk-sac atrophies towards the end of the first trimester and after this synthesis is by the fetal liver. The two sources produce different forms of AFP (Ruoslahti *et al.*, 1978) which can be differentiated by their binding to concavalin A, fetal liver AFP having much greater affinity. At 17 weeks of pregnancy the concentration of AFP in fetal serum is 50,000 times greater than in maternal serum (Wald and Cuckle, 1984). AFP is the fetal equivalent of serum albumin.

AFP levels, measured prospectively at the time of sample collection as part of routine screening, were available for all cases. AFP levels were determined using a radioimmunoassay until June 1985. Thereafter, the method was changed to an immunoradiometric assay (Stevenson *et al.*,1987).

4.1.2.1 RADIOIMMUNOASSAY FOR ALPHAFETOPROTEIN

A semi-automated protocol using a Micromedic APS-2 sample processor to dispense assay buffer and serum was employed. 40 µl of sample or standard serum were incubated with 800µl assay diluent (0.05 mol/l barbitone, pH 8.6, with 0.1% w/v sodium azide)

containing 10% w/v polyethylene glycol 6000 (BDH), 1.1 µg/l ^{125}I-AFP 310 µg/l, bovine gamma globulin (Sigma) and sheep anti-human AFP antiserum (final dilution 1:52,000) for 16 hours at ambient temperature. All samples were assayed in duplicate. Bound and free fractions were separated by centrifugation at 1,500g for 30 minutes at 4°C. The supernatant was then aspirated. The bound fraction was counted on an LKB Rackgamma counter. Data processing used the programme developed by McKenzie and Thomson (1983), which uses 5 parameter logistics for curve fitting, in a PDP11/24 minicomputer (DEC).

4.1.2.2 IMMUNORADIOMETRIC ASSAY FOR ALPHAFETOPROTEIN

This is a two-site IRMA. Samples and assay buffer were dispensed using a Micromedic APS-2 or Kemble 1000 automatic sample processor. 25µl of serum sample or standard were diluted with 200µl assay buffer (0.1 mol/l EPPS (Sigma), pH 8.0, 0.1% v/v Tween 20 (Sigma), and 0.1% w/v sodium azide (Sigma)). To this was added 200 µl assay buffer containing 2.2% v/v sheep serum (SAPU), 25 µg/l ^{125}I monoclonal anti-AFP (AF5/A2; Radioimmunoassay Section, Department of Obstetrics and Gynaecology, Ninewells Hospital Dundee) and 5 g/l polyclonal sheep anti-human AFP linked to Sepharose CL-4B (SAPU). All samples were assayed in duplicate. The assay was incubated at ambient temperature for 2½ hours on an orbital shaker at 400 rpm. Separation of bound and free fractions was by two cycles of sucrose density sedimentation. First 1 ml of wash reagent (9 g/l sodium chloride (BDH) and 0.1% v/v Tween 20) was added to each tube. Then 3 ml of density sedimentation reagent

(30 g/l sucrose, 0.1% v/v Tween 20) was pumped carefully into the bottom of each tube. After 15-30 minutes the supernatant was aspirated and after the process had been repeated, the bound fraction counted in either an LKB Rackgamma counter or a Packard Cobra 5010 gamma counter. Data reduction was either by the method described for the RIA AFP assay or, using an Epson PC AX microcomputer, by the WHO immunoassay programme developed by Edwards and E kins (1983) which uses a 4 parameter mass action model for curve fitting.

4.1.2.3 AFP ASSAY PARAMETERS

The RIA assay had a working range of 25-400 KU/l and typically had an inter-assay coefficient of variation (CV) of 11% and an intra-assay CV of 5% between 40-400 KU/l. The IRMA assay has a working range of 1.5-500 KU/l and typically has an inter-assay CV of 6.0% and intra-assay CV of 2.5% between 5 and 500 KU/l. The AFP results for both RIA and IRMA methods were expressed as KU/l (IRP 72/227) and converted to multiples of the median (MOM) by using the appropriate median for unaffected pregnancies for the gestation and assay method.

4.1.3 HUMAN CHORIONIC GONADOTROPHIN

HCG is a sialoglycoprotein with a molecular weight of 40,000, produced in the syncytiotrophoblast of the placenta (Dreskin *et al.*, 1970). It is composed of two non-covalently bound subunits

of unequal size, α and β (Bahl *et al.*, 1972). The α subunit is virtually identical to that of the pituitary hormones luteinising hormone (LH), follicle-stimulating hormone (FSH), and thyroid stimulating hormone (TSH) (Bahl *et al.*, 1972). The β subunit shares a high degree of homology with LH, but possesses an extra 24 amino acid carboxy-terminal extension. The βLH and βhCG subunits can be distinguished immunologically (Vaitukaisis *et al.*, 1972). The major physiological role of hCG appears to be support of corpus luteum progesterone production, a function essential for pregnancy maintenance in the first seven weeks (Csapo *et al.*, 1973). The concentration of hCG in the maternal circulation far exceeds that found in the fetal circulation (Vaitukaisis, 1977).

4.1.3.1 IMMUNORADIOMETRIC ASSAY FOR HUMAN CHORIONIC GONADOTROPHIN

For both retrospective and prospective studies hCG was estimated using a commercially available immunoradiometric assay (Serono MAIA-clone kit) which measures predominantly intact molecule hCG but does have some cross-reactivity with free β subunit of hCG. This assay utilizes three monoclonal antibodies to hCG, two of which are labelled with ^{125}I. The third monoclonal, recognising a different epitope on the hCG molecule, is labelled with fluorescein. After incubation, sheep antiserum to fluorescein coupled to a magnetic solid phase is added in excess. This rapidly and specifically binds to the hCG-monoclonal antibody complex and is sedimented in a magnetic field.

For retrospective studies abnormal cases and their matched

controls were randomised and assayed in the same batch. To bring second trimester serum samples into the range of the assay all samples were diluted 1 in 500 in a two-step dilution (1/20 then 1/25) using the horse serum supplied with the kit. For reasons of economy all sample, standard and reagent volumes, apart from the final wash step, were halved from that given in the supplied protocol. This change did not appear to alter the performance of the assay. The sensitivity remained unchanged at 0.5 mIU/ml and over four assays the inter-assay CVs for two quality control samples with values of 78 IU/ml and 27 IU/ml were 7.8% for assay done with full volume and 7.4% for assays at half volume.

25 µl of standard or diluted sample were pipetted into tubes. All standards and samples were assayed in duplicate. 250 µl of ^{125}I anti-hCG reagent, containing fluorescein and ^{125}I labelled mouse monoclonal antibodies to hCG in Tris buffer with normal sheep serum, bovine serum albumin (BSA) and 0.2% w/v sodium azide, was added to each tube. The tubes were vortex mixed and incubated at 37°C in a water bath for 15 minutes. 100µl of separation reagent, containing sheep antiserum to fluorescein covalently bound to magnetic particles in Tris buffer with BSA and 0.1% w/v sodium azide, was added to each tube and, after gentle mixing, incubated at room temperature for 5 minutes. The racks of tubes were placed in magnetic separators and the particles allowed to sediment for 2 minutes. The supernatant was decanted by inversion of the rack. A final wash step reduces non-specific binding. 500µl of wash buffer (Tris buffer with 0.2% w/v sodium azide) was added to each tube and all tubes thoroughly mixed by vortexing. The magnetic sedimentation step and decanting of the supernatant were repeated.

The bound fraction was counted using a Packard Cobra 5010 gamma counter. Data reduction was by either the data reduction package in the gamma counter on-board microcomputer using spline curve fitting on a log/linear scale (RIA-Smart) or by the WHO package described in the AFP IRMA assay method.

For routine use in the whole screening population, a semi-automated hCG assay protocol was used, with the assay dilution step, and the dispensing of sample being carried out by a Kemble 1000 automatic sample processor.

4.1.3.2 hCG ASSAY PARAMETERS

The assay has a range of 0.5-500 mIU/ml (0.25-250 IU/ml for 1 in 500 diluted samples). The hCG results were expressed as IU/ml (first IRP 75/537) and converted to multiples of the median of the appropriate gestation. Median values for each gestation for the controls were calculated by linear interpolation. Two quality control samples, with values of 29 and 81 IU/ml were assayed twice in each batch. For the retrospective manual assays the inter-assay CV was 8.3% and the intra-assay CV 2.6% at 29 IU/ml and 6.2% and 2.6% at 81 IU/ml. The routine semi-automated assay typically has an inter-assay CV of 7.5% and an intra-assay CV of 3% between 5 and 100 IU/ml.

4.1.4 PREGNANCY SPECIFIC β-1 GLYCOPROTEIN

SP_1 is a glycoprotein with a molecular weight of 90,000. It is synthesised by the syncytiotrophoblast of the placenta (Horne *et al.*, 1976). Circulating levels of SP_1 rise steadily during gestation and reach a plateau near term (Bohn, 1980). The maternal levels of SP_1 are higher in molar terms than any other placental product (Chard and Grudzinskas, 1980). Levels of SP_1 found in the maternal circulation greatly exceed those found in the fetal circulation (Gordon and Chard, 1979).

4.1.4.1 PREGNANCY SPECIFIC β-1 GLYCOPROTEIN ASSAY

SP_1 concentration was measured by 'Rocket' immunoelectrophoresis (Teisner *et al.* 1978), using commercially available β-1 glycoprotein antiserum (Dakopatts) and, as a standard, lyophilised β-1 glycoprotein (Behring). Agarose gels were prepared on 8cm x 8cm glass plates using 10 ml of 1% w/v Agarose 15 (BDH) in Barbitone buffer (3mmol/l diethyl barbituric acid (BDH), 17 mmol/l sodium barbitone (BDH) and 1.7mmol/l calcium lactate (BDH), pH 8.6). The central strip of agarose (2cm wide) was cut out and replaced with 2.5 ml buffered agarose containing anti-SP_1 antiserum at an antibody dilution of 1:62.5. A line of wells was cut in the agarose below the antibody strip. Abnormal cases and their matched controls were selected blind. A lyophilised β-1 glycoprotein preparation (Behring), reconstituted to a concentration of 48.4 mg/L, was used as a standard. 2 µl of sample or standard was added to each well. Samples and standards were

assayed in quadruplicate, using pairs of wells on two separate plates. The plates were placed in an electrophoresis tank prepared with Barbitone electrophoresis buffer (7.5 mmol/l diethyl barbituric acid (BDH), 42.5 mmol/l sodium barbitone (BDH) and 1.2 mmol/l calcium lactate (BDH), pH 8.6) and electrophoresed at constant voltage (90 volts, 6-10 mAmps) at 4°C overnight. SP_1 migrates from the cathode to the anode. The plates were rinsed with distilled water, dried using filter paper overlays in a warm air stream and stained with Coomassie brilliant Blue (2 g/l Coomassie Brilliant Blue (BDH) in 45% v/v methanol (BDH), 10% v/v glacial acetic acid) and dried again. Peak heights were measured in millimetres and the concentration of SP_1 calculated from the height of the standard peak by proportion.

4.1.4.2 SP_1 ASSAY PARAMETERS

A quality control sample, prepared from pooled maternal serum at 16-21 weeks, and with value of 23.0 mg/l, was run on each plate. The inter-assay CV was 15.3% and the intra-assay CV 8.8%. SP_1 results were expressed as mg/L and converted to multiples of the median for the appropriate gestation. Median control values were calculated for each gestation by weighted linear regression.

4.1.5 UNCONJUGATED ESTRIOL

UE3 is a steroid hormone which is virtually undetectable in non-pregnant women. It is produced principally from placental

conversion of fetal 16α-hydroxy-dehydroepiandrosterone sulphate (16α-DHEAS) (Diczfalusy, 1974). 16α-DHEAS is produced in the fetal liver from dehydroepiandrosterone sulphate (DHEAS). DHEAS is synthesised in the fetal adrenal cortex from cholesterol (Buster, 1984). Approximately 90% of UE3 produced in the fetoplacental unit is derived from fetal DHEAS and only 10% from maternal DHEAS (Siiteri and MacDonald, 1963). In the maternal circulation 5-10% of estriol is present in the unconjugated form and the rest as conjugates of sulphate and glucuronide, the most abundant form being estriol-3-sulphate, -16-glucuronide (Levitz *et al.*, 1975). Concentrations of circulating unconjugated estriol are 4-8 times higher in the fetus than in the maternal circulation (Pasqualini and Kincl, 1985).

4.1.5.1 UNCONJUGATED ESTRIOL ASSAY

UE3 was measured using a commercially available radioimmunoassay (Amersham AMERLEX-M, IM4, 2nd trimester). This assay kit is optimised for use at the concentration levels of UE3 prevailing in the second trimester, with a range of 1-50 nmol/l, and replaces the Amersham IM2 assay designed for use at the higher third trimester concentrations. Unlike the previous third trimester UE3 assay the optimised 2nd trimester assay requires no modification to increase its sensitivity and has a zero standard which is charcoal stripped human serum and should contain no UE3. The anti-UE3 antibody is bound to magnetic polymer particles and separation of the antibody bound fraction is by magnetic separation.

For economy of sample volume the assay method was modified to use half the stated volume of reagents, standards and samples. This change in protocol did not alter the sensitivity of the assay from 0.2 nmol/l. The intra-assay CVs also remained similar, with CVs for four samples with values of 1.9, 4.0, 8.2 and 30.8 nmol/l of 7.6%, 6.0%, 7.2% and 6.2% in 10 duplicates in a full volume assay, and 7.5%, 6.2%, 4.8% and 7.3% in a half volume assay. Abnormal cases and their matched controls were randomised and assayed in the same batch. 40 µl of sample or standard were pipetted into tubes. All standards and samples were assayed in duplicate. 40 µl of ^{125}I labelled estriol solution and 400 µl of AMERLEX-M estriol antibody suspension (containing anti-UE3 bound to magnetic particles) were added to each tube and incubated at 37°C in a water bath for 1 hour. The racks of tubes were then attached to the magnetic separator at room temperature and the magnetic particles allowed to sediment for 15 minutes. The supernatant was discarded by inversion of the tubes. The bound fraction was counted using a Packard Cobra 5010 gamma counter. Data reduction was performed by the gamma counter on-board microcomputer, using spline curve fitting on a log/linear scale (RIA-Smart).

4.1.5.2 UE3 ASSAY PARAMETERS

The UE3 results were expressed as nmol/l and converted to multiples of the median for the appropriate gestation. Median values for each gestation for the controls were calculated by

linear interpolation. Four quality control samples, with values of 1.9, 3.9, 8.0, 27.6 nmol/l were assayed twice in each batch. The inter-assay CVs were 6.8%, 5.6%, 5.6% and 7.2% respectively and the intra-assay CVs, 5.8%, 4.9%, 3.5% and 5.4% respectively.

4.1.6 FREE ß SUBUNIT OF hCG

In the second trimester of pregnancy, maternal serum levels of unbound ß subunit hCG are around 0.5% of the levels of the intact (α + ß) molecule (Osturk *et al.*, 1988). The use of monoclonal antibodies has led to the ability to construct assays of greater specificity. Knowledge of the stereo structure of the hCG molecule and advances in raising epitope-specific assays have resulted in the production of antibodies capable of recognising free α subunit, free ß subunit, C-terminal region and intact hCG (Norman *et al.*, 1990). Assays for Frß-hCG recognise an epitope on the ß subunit which is on the binding site of the α and ß subunits and which is therefore not exposed in the intact molecule (Macri *et al.*, 1990, Macri *et al.*, 1993).

4.1.6.1 FREE ß SUBUNIT OF hCG ASSAY

The free ß-subunit of hCG (Frß-hCG) was measured using a commercially available immunoradiometric assay (Bioclone Free ß hCG kit) which measures predominantly free ß hCG but has a small amount of cross-reactivity with intact hCG (up to 0.4%). The free ß subunit to intact molecule ratio in the maternal circulation in

the second trimester of pregnancy is around 1:200. The assay utilizes two monoclonal antibodies, one labelled with ^{125}I and the other bound to magnetised polystyrene particles, directed against different free ß subunit epitopes.

For economy of sample the assay method was modified to use half the volume of samples, standards and reagents. This did not appear to change the performance of the assay. The sensitivity remained unchanged at 0.3 IU/l. Two samples, run in five different assays, had inter-assay CVs of 14.3% for the full volume assay and 13.8% for the half volume assay at 6.4 IU/l and 7.3% for the full volume assay and 6.9% for the half volume assay at 88 IU/l. Abnormal cases and their matched controls were randomised and assayed in the same batch. 40 µl of sample or standard were pipetted into tubes. All standards and samples were assayed in duplicate. 200 µl of the solution containing ^{125}I anti-free ß hCG (in BSA PBS buffer with 0.1% w/v sodium azide) and 200 µl of the suspension of anti-free ß hCG bound to magnetic particles (in BSA PBS buffer with 0.1% w/v sodium azide) were added to each tube. The tubes were vortexed gently then incubated at 37°C in a water bath for 1 hour. The racks of tubes were then placed in magnetic separators and the particles allowed to sediment for 15 minutes. The supernatant was decanted by inversion of the rack. A final wash step reduces non-specific binding. 200µl of wash buffer (BSA PBS buffer with 0.1% w/v sodium azide) was added to each tube and all tubes thoroughly mixed by vortexing. The magnetic sedimentation and decanting of the supernatant steps were repeated. The bound fraction was counted using a Packard Cobra 5010 gamma counter. The average CPM for each pair of duplicates was determined. The

standard curve was plotted on log-log graph paper and the results for the samples read from the graph.

4.1.6.2 FREE β SUBUNIT ASSAY PARAMETERS

The assay has a range of 0.5-500 IU/l. The hCG results were expressed as IU/l (IRP 75/551) and converted to multiples of the median of the appropriate gestation. Median values for each gestation for the controls were calculated by linear interpolation. Two quality control samples, with values of 3.9 IU/l and 20.5 IU/l were assayed twice in each batch. The inter-assay CV was 22.8% and the intra-assay CV was 16.7% at 3.9 IU/l and 7.0% and 4.1% at 20.5 IU/l.

4.2 STATISTICAL METHODS

4.2.1 MEDIAN AND CENTILES

The median is defined as the middle value of the variable, when the data are arranged in order of increasing magnitude. It can be calculated from a frequency distribution, where the values have been divided into classes of even width, using the formula below and is the ((n+1)/2)th value.

$$\text{Median} = \left[\frac{(P - f)}{(F - f)} \times (X - x)\right]$$

Where X = upper boundary of median class

x = lower boundary of median class

F = upper frequency of median class

f = lower frequency of median class

P = median frequency i.e. (n + 1)/2

Medians can also be generated from a frequency distribution derived using a statistical calculation programme in a microcomputer. SPSS/PC$^+$ was used for many of the calculations.

Similarly, particular centiles can be derived from a set of data. The *p*th centile is the value of the variable such that *p* percent of the measurements are less than that value and (100 - *p*) are greater. Centiles were calculated either by using the same method as for the median, working out the *p*th centile as the (n-*p*)/100 th value, or using the SPSS/PS$^+$ statistical package.

4.2.1.1 MEDIANS BY WEIGHTED LOG-LINEAR REGRESSION

This is a method of smoothing the median values obtained from the raw data, where less than an optimum number of values are available for each gestational week, and where the medians obtained directly from the raw data are erratic.

Medians were first calculated for each week of gestation from the raw data and then log-linear regression analysis, weighted according to the number of data points at each gestational age, was carried out to obtain the equation of the regression line. Smoothed medians were then calculated from the equation and used to work out multiples of the median. The calculation was as follows:

$$\text{Regressed median} = 10^{(MG + B)}$$

$$M = \text{slope} = N/D$$

$$B = \text{intercept} = \bar{Y} - M\bar{G}$$

$$N = \Sigma W(G - \bar{G})(\log Y - \bar{Y})$$

$$D = \Sigma W(G - \bar{G})^2$$

$$\bar{Y} = \frac{\Sigma(\log Y \,.\, W)}{\Sigma W}$$

$$\bar{G} = \frac{\Sigma(G \,.\, W)}{\Sigma W}$$

where G = gestation (weeks)

Y = raw median

$\log Y = \log_{10}(\text{raw median})$

W = number of samples at week of gestation.

Substituting the required median into the derived formula for the

regressed median gives the value of the regressed median.

4.2.2 GAUSSIAN DISTRIBUTIONS

A Gaussian or normal distribution is a symmetrical bell-shaped curve with the formula:

$$y = \frac{1}{SD\sqrt{(2\pi)}} \cdot e^{-\frac{1}{2}\left[(x-\bar{x})/SD\right]^2}$$

where $\bar{x}$ = mean

SD = standard deviation

The curve is symmetrical about the mean.

4.2.3 MEAN AND STANDARD DEVIATION

The mean ($\bar{x}$) is a measure of central tendency, that is, a measure of the central point of the distribution of data. It was calculated by:

$$\bar{x} = \frac{\sum_{n=1}^{n} x}{n}$$

where n = number of measurements

The standard deviation (SD) is a measure of the variability or dispersion of the data. It was calculated by:

$$SD = \frac{\sum_{n=1}^{n} (x - \bar{x})^2}{n-1}$$

The standard deviation represents the horizontal distance on either side of the mean which reaches out to the points of inflection i.e. the points on the Gaussian curve where the slope changes from getting steeper to getting flatter. Means and standard deviations were calculated using a pocket calculator with statistical functions or using the SPSS/PC+ statistical package in a microcomputer.

4.2.3.1 ESTIMATION OF THE MEAN AND STANDARD DEVIATION FROM A FREQUENCY DISTRIBUTION

Means and standard deviations were also estimated from a frequency distribution as described by Cuckle *et al.* (1987). For a Gaussian distribution the median was taken as an estimate of the mean and the standard deviation estimated from the difference between the 90th and 10th centiles, divided by 2.56. For a log Gaussian distribution $\log_{10}$(Median) was taken as an estimate of the mean and the difference between $\log_{10}$(90th centile) and $\log_{10}$(10th centile), divided by 2.56, taken as an estimate of the standard deviation. This method avoids undue influence of outlying values when relatively small numbers of values are being used.

4.2.4 PROBABILITY PLOTS

Before using many statistical tests and also when using data for deriving risks (see Section 4.2.8) it is important to know whether data can reasonably be regarded as sampled from an underlying

Gaussian distribution or can be transformed i.e. by taking $\log_{10}$, to fit a Gaussian distribution. One way of proving this graphically is by a probability plot.

For a given set of data, all values were ranked in ascending order. Each value was assigned its centile value and from these, using the known area under a Gaussian distribution (available in statistical tables) the standard deviation of each value derived. A graph was plotted with the standard deviations on a linear scale on the x-axis and the values on either a linear scale (for a Gaussian distribution) or a $\log_{10}$ scale (for a log Gaussian distribution) on the y-axis. A continuous line of the appropriate Gaussian or log Gaussian distribution derived from the mean and standard deviation of the data was also plotted and the graph inspected for deviation from the expected distribution.

4.2.5 STUDENT'S t-TEST

This is used to compare two different groups, each with known mean and standard deviation, to test whether they should be regarded as (a) samples from two different underlying populations, or (b) samples from one underlying population, the difference between means having arisen by chance. The t-test should only be used on data shown to be normally distributed.

The statistic t can be calculated by varying formulae, depending on whether the data are paired or unpaired and whether the two samples have the same or differing standard deviations. The

formula below, which is for unpaired variables and two samples with differing standard deviations was used.

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{(((n_1 \times SD_1) + (n_2 \times SD_2))/(n_1 + n_2 - 2))} \times \sqrt{(1/n_1 + 1/n_2)}}$$

Degrees of freedom = $n_1 + n_2 - 2$

The null hypothesis for the t-test is that there is no difference in the underlying populations from which the two samples were drawn, and that the differences in mean are due to chance. Statistical tables, at the appropriate degrees of freedom, were consulted for the calculated t values and the probability value associated with the found value of t derived. If the probability (p) is <0.05 or <0.01 the difference between the two means is said to be significant and the null hypothesis rejected.

4.2.6 KOLMOGOROV-SMIRNOV TEST

The Kolmogorov-Smirnov (K-S) test is used to test the deviations of observed frequency distributions from expected ones. The K-S statistic, D, is defined as the absolute value of the largest difference between two cumulative frequency distributions, one expected and the other observed. Each value in the distribution being tested is converted to a standard deviation. From these, using statistical tables, the cumulative area under the curve to each value is derived. These are compared with the predicted cumulative frequency distribution and the difference between these at each individual value calculated. The value of D is the largest

absolute difference found. The found value of D can be looked up in statistical tables at the appropriate sample size and the probability (p) derived. The null hypothesis is that there is no difference between the expected and observed distributions. If the probability (p) is less than 0.05 or 0.01 the difference between the observed and expected distributions is said to be significant and the null hypothesis rejected. The SPSS/PC+ statistical package was used for the majority of the calculations of the K-S statistic (D) and the associated probability.

4.2.7 CORRELATION

For two sets of normally distributed data the product-moment correlation coefficient (r) can be calculated to give a measure of the level of association between the variables. For two variables, x and y, the formula for r is

$$r = \frac{\Sigma(x - \bar{x})(y - \bar{y})}{\sqrt{(\Sigma(x - \bar{x})^2 \cdot \Sigma(y - \bar{y})^2)}}$$

The correlation coefficient , r, has values from 0 to ±1. A positive value of r signifies positive correlation and a negative value negative correlation. Values of r close to 0 indicate virtually no association between variables and the closer the value of r gets to 1 the greater the level of association.

To determine statistical significance, the calculated value of r was looked up in statistical tables at the appropriate degrees of freedom (n - 2) and the probability (p) derived from the table.

The null hypothesis is that there is no association between the variables.

Values of r and the appropriate significance were mainly calculated using the SPSS/PC+ statistical package.

4.2.8 DERIVING RISKS (LIKELIHOOD RATIOS) FROM GAUSSIAN DISTRIBUTIONS

The risk (likelihood ratio) that a specific result of a particular analyte is associated with an abnormality, such as Down's syndrome, is the proportion of affected pregnancies with the given analyte level, divided by the proportion of unaffected pregnancies with the same level. It is estimated as the height to the Gaussian or log Gaussian distribution of affected pregnancies divided by the height to the distribution of unaffected pregnancies and can be calculated by the formula

$$LR = (SD_C/SD_D)\ e^{-\frac{1}{2}\left[\left[\frac{\log_{10}(x) - M_D}{SD_D}\right]^2 - \left[\frac{\log_{10}(x) - M_C}{SD_C}\right]^2\right]}$$

where M_D and SD_D are the mean and standard deviation of the affected distribution and M_C and SD_C the mean and standard deviation of the control distribution (Palomaki and Haddow, 1987, Cuckle *et al.*, 1987). Likelihood ratios, and hence the risk of an affected pregnancy, increase moving towards the affected curve and decrease moving towards the unaffected curve. At the point of

intersection of the two curves the likelihood ratio is equal to one.

Likelihood ratios can also be calculated for two variables by a combined formula which allows for correlation between the two (Wald *et al.*, 1988a). In this case the formula for calculating the combined likelihood ratio at a given level (x) for analyte (1) and (y) for analyte (2) is

$$LR = \frac{{}^{1}SD_C \; {}^{2}SD_C}{{}^{1}SD_D \; {}^{2}SD_D} \cdot \sqrt{\frac{(1 - (R_C)^2)}{(1 - (R_D)^2)}} \cdot e^{\left[\frac{V_C - V_D}{2}\right]}$$

$$V_C = \frac{(({}^{1}Z_C)^2 - 2R_C{}^{1}Z_C{}^{2}Z_C + ({}^{2}Z_C)^2)}{(1 - (R_C)^2)}$$

$$V_D = \frac{(({}^{1}Z_D)^2 - 2R_D{}^{1}Z_D{}^{2}Z_D + ({}^{2}Z_D)^2)}{(1 - (R_D)^2)}$$

$${}^{1}Z_C = \frac{\log_{10}(x) - {}^{1}M_C}{{}^{1}SD_C}$$

$${}^{1}Z_D = \frac{\log_{10}(x) - {}^{1}M_D}{{}^{1}SD_D}$$

$${}^{2}Z_C = \frac{\log_{10}(y) - {}^{2}M_C}{{}^{2}SD_C}$$

$${}^{2}Z_D = \frac{\log_{10}(y) - {}^{2}M_D}{{}^{2}SD_D}$$

where, for analyte (1), ${}^{1}M_C$ and ${}^{1}SD_C$ are the mean and standard deviation of the control distribution and ${}^{1}M_D$ and ${}^{1}SD_D$ those of the affected distribution, and, for analyte (2), ${}^{2}M_C$ and ${}^{2}SD_C$ are the mean and standard deviation of the control distribution and ${}^{2}M_D$ and ${}^{2}SD_D$ those of the affected distribution. R_C is the

correlation coefficient between analyte (1) and analyte (2) for the controls and R_D that for the affected samples.

4.2.9 CALCULATION OF DETECTION AND FALSE POSITIVE RATES

The proportion of women at each individual maternal age in the west of Scotland screened population was derived from the ages of women having an AFP screening test during 1986 and 1987, adjusted for the number of women aged 35 years and over proceeding directly to amniocentesis without having a screening test.

The proportion of Down's syndrome or autosomal trisomy pregnancies at each individual maternal age was derived by calculating, from the number of women and the maternal age risk, the number of affected pregnancies per year at each maternal age in the west of Scotland population. These were then converted to percentages.

Likelihood ratios for each analyte or combination of analytes were calculated for all of the affected and control samples. The distributions of these likelihood ratios were then used to estimate detection and false positive rates. The detection rate at a specific risk threshold for a particular combination of analytes was calculated at each individual maternal age from the proportion of the distribution of the likelihood ratios for the affected samples which would give a risk equal to or greater than the specified risk. This was then multiplied by the predicted percentage of affected pregnancies at each maternal age and an overall detection rate obtained by summation. Similarly, the

corresponding false positive rate was obtained using the distribution of likelihood ratios for the control samples and the percentage of pregnancies at individual maternal ages in the west of Scotland pregnant population.

4.2.10 CONFIDENCE LIMITS

4.2.10.1 95% CONFIDENCE LIMITS OF THE MEAN

The mean obtained from a series of measurements is a point estimate of the mean of the underlying population. To obtain an interval estimate of the underlying mean lower and upper boundary values can be calculated within which the population mean has a 95% probability of falling. These are 95% confidence limits (CL). The formula for calculating these is

$$95\%\ CL = \bar{x} \pm t(SEM)$$

where t = Students t statistic at the appropriate degrees of freedom (n-1)

SEM = standard error of the mean

$$SEM = \frac{SD}{\sqrt{n}}$$

Where medians were used as estimates of the mean, a 95% confidence interval was calculated for the mean and used as a confidence interval for the median, taking 10^x if from a log Gaussiasn distribution.

4.2.10.2 95% CONFIDENCE INTERVALS FOR SINGLE OBSERVED VALUES

The Poisson distribution is a discrete frequency distribution of the number of times a rare event occurs. The events must be random and occur independently of each other. For observed values which fulfil these criteria, the range which represents 95% confidence limits was obtained from the appropriate statistical tables.

4.2.10.3 95% CONFIDENCE INTERVALS FOR PROPORTIONS AND PERCENTAGES

Confidence intervals for proportions or percentages were derived from statistical tables based on the binomial distribution, which is the probability distribution of random discrete variables with two possible outcomes. For sample numbers greater than 100, the binomial distribution approaches the normal distribution and the 95% confidence interval was calculated from the formula:

$$p \pm \sqrt{(p(p - 1)/n)}$$

where p is the proportion.

SECTION 5

RESULTS

PHASE 1

5.1 MATERNAL SERUM ALPHAFETOPROTEIN AND CHROMOSOME ABNORMALITIES

5.1.1 RETROSPECTIVE ANALYSIS OF AFP LEVELS

5.1.1.1 AFP LEVELS IN UNAFFECTED PREGNANCIES

Maternal serum AFP results, stored on a database between April 1982 and May 1987, from 113,045 singleton pregnancies unaffected by neural tube defect or chromosome abnormality were analysed to provide normal population parameters. Median AFP values increase with advancing gestation between 16 and 20 weeks. For the RIA assay (prior to July 1985, 69,000 samples) median values were 27 KU/l at 16 weeks, 31 KU/l at 17 weeks, 36 KU/l at 18 weeks, 42 KU/l at 19 weeks and 49 KU/l at 20 weeks. For the IRMA assay (July 1985 onwards, 44,000 pregnancies) median values were 34 KU/l at 16 weeks, 37 KU/l at 17 weeks, 43 KU/l at 18 weeks, 50 KU/l at 19 weeks and 58 KU/l at 20 weeks. The appropriate method-related gestational median was used to convert AFP levels in individual autosomal trisomy pregnancies to MOM.

5.1.1.2 AFP LEVELS IN AUTOSOMAL TRISOMIES

Over the same screening period 142 autosomal trisomy pregnancies were also identified for which maternal serum AFP results were available. Individual maternal serum AFP levels (in MOM) for each pregnancy with an autosomal trisomy plotted at the appropriate completed week of gestation are shown in Figure 5-1. The median AFP value for the autosomal trisomy pregnancies was significantly

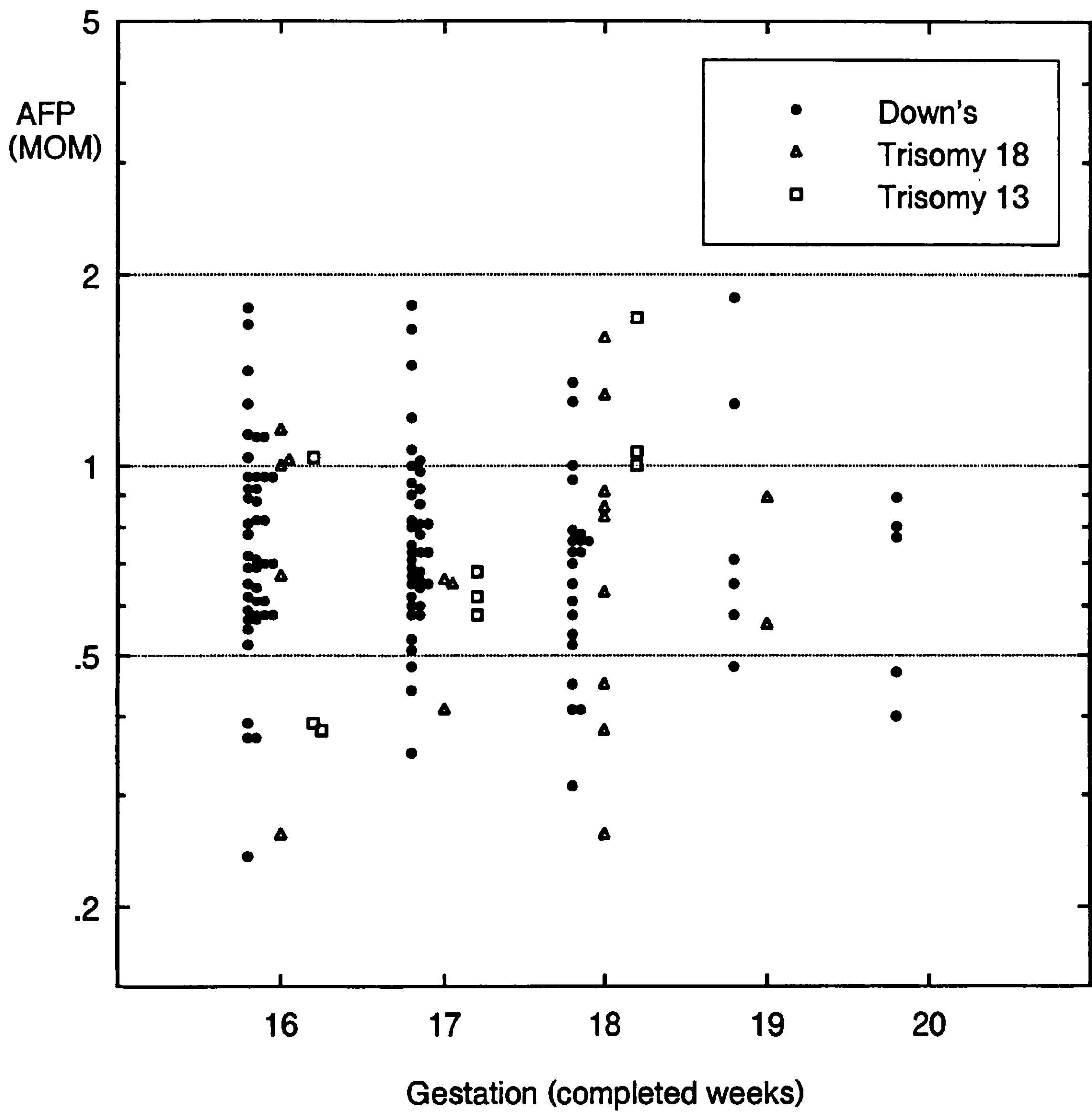


Figure 5 - 1

Maternal serum alphafetoprotein (AFP) levels, in multiples of the median (MOM), in 142 autosomal trisomy pregnancies (114 Down's, 19 Trisomy 18, 9 Trisomy 13) compared with the levels in 113,045 unaffected singleton pregnancies.

lowered at 0.72 MOM (95% CL 0.67 - 0.77 MOM) of the unaffected pregnancies ($p<0.001$ by t-test on unpaired variables). The proportions of control, Down's syndrome, trisomy 18 and 13 and all autosomal trisomy pregnancies with values less than or equal to selected cut-off levels are shown in Table 5-1 with, for example, 117 out of 142 autosomal trisomy values (82%) equal to or below the median. Also 12% of pregnancies with Down's syndrome, 25% of trisomies 18 and 13 and 15% of all autosomal trisomies had AFP levels less than 0.5 MOM compared with 4.2% of unaffected pregnancies. Probability plots (Figure 5-2) show that the AFP levels in the unaffected pregnancies Figure 5-2a), the Down's syndrome pregnancies (figure 5-2b), the trisomies 18 and 13 combined (Figure 5-2c) and the entire group of autosomal trisomies (Figure 5-2d) fit log Gaussian distributions between 0.35 and 2.0 MOM (Figure 5-2). The log Gaussian distributions of the unaffected and autosomal trisomy distributions are shown in Figure 5-3. The intersection point of the two curves is 0.85 MOM There was no statistically significant difference between the means for the trisomy 18 group (0.68 MOM) and the trisomy 13 group (0.74 MOM, t-test, $p>0.5$) nor between the trisomies 18 and 13 combined (0.72 MOM) and the Down's syndrome cases (0.72 MOM, t-test, $p>0.5$). The means and standard deviations of the controls, Down's and autosomal trisomy distributions were estimated as described in Section 4.2.3.1 and are shown in Table 5-2. There was no evidence of correlation between AFP and age ($r=0.0098$, $p>0.05$) in the Down's syndrome pregnancies.

Table 5-1

Proportions of unaffected singleton pregnancies, Down's syndrome pregnancies and autosomal trisomy pregnancies with alphafetoprotein (AFP) levels, in multiples of the median (MOM) equal to or less than selected cut-off levels.

AFP (MOM)	Unaffected (n=113,045) % (n)	Down's (n=114) % (n)	Trisomy 18+13 (n=28) % (n)	Trisomy 21+18+13 (n=142) % (n)
≤1.6	89 (100,972)	96 (109)	96 (27)	96 (136)
≤1.4	82 (92,622)	94 (107)	93 (26)	94 (133)
≤1.2	70 (78,665)	90 (103)	89 (25)	90 (128)
≤1.0	52 (58,119)	84 (96)	75 (21)	82 (117)
≤0.9	39 (44.576)	74 (84)	64 (18)	72 (102)
≤0.8	28 (31,276)	64 (73)	53 (15)	62 (88)
≤0.7	18 (20,059)	46 (53)	50 (14)	47 (67)
≤0.6	9 (10,597)	28 (32)	32 (9)	29 (41)
≤0.5	4 (4,752)	12 (14)	25 (7)	15 (21)
≤0.4	2 (1,890)	6 (7)	18 (5)	8 (12)

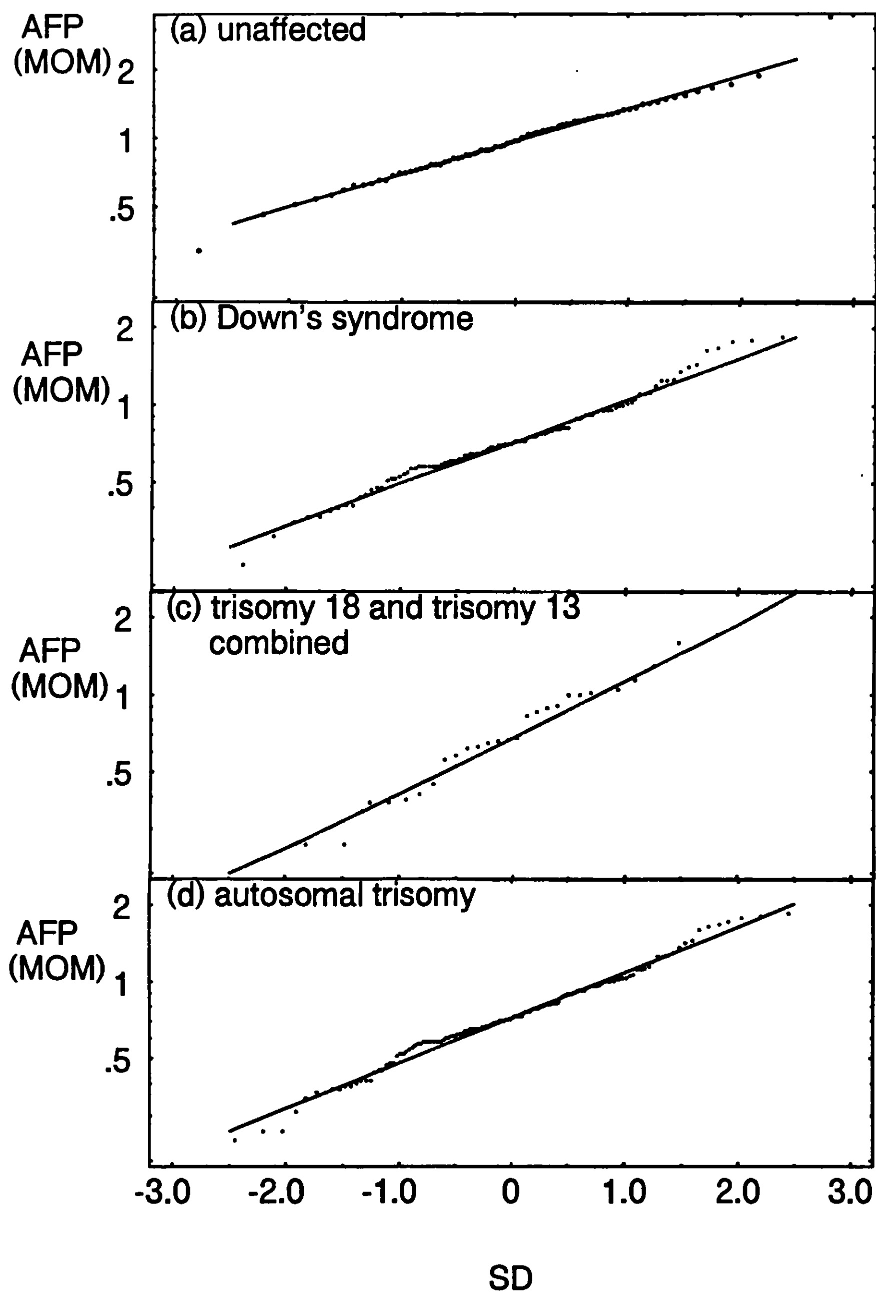


Figure 5 - 2

Probability plot of maternal serum alphafetoprotein (AFP) levels, in multiples of the median (MOM), on a log scale, in (a) a sub-group of 113,000 unaffected pregnancies, (b) 114 Down's syndrome pregnancies, (c) 28 trisomy 18 and 13 pregnancies, (d) all 142 autosomal trisomies combined. The continuous lines are those defined by log Gaussian distributions with means and standard deviations as given in Table 5-2.

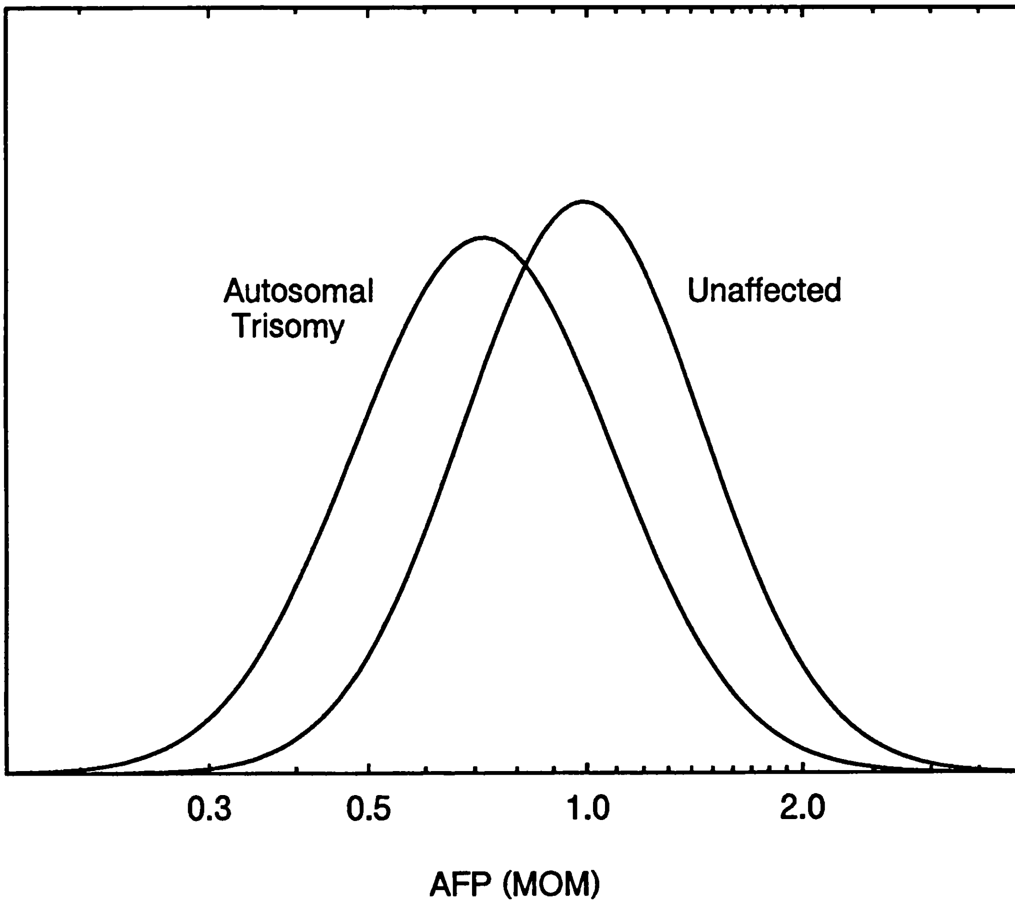


Figure 5 - 3

Log Gaussian distributions of maternal serum alphafetoprotein (AFP) levels, in multiples of the median (MOM), plotted from the means and standard deviation of 113,000 unaffected and 142 autosomal trisomy pregnancies as given in Table 5-2.

Table 5-2

Medians, means and standard deviations of the maternal serum alphafetoprotein (AFP) distributions in Down's syndrome, autosomal trisomy and unaffected pregnancies.

	(n)	Median (MOM)	Mean $\log_{10}$	SD $\log_{10}$
Down's syndrome	114	0.72	-0.1427	0.1626
Trisomy 18	19	0.68	-0.1673	0.2201
Trisomy 13	9	0.74	-0.1304	0.2159
Trisomy 18+13	28	0.70	-0.1549	0.2190
Trisomy 21+18+13	142	0.72	-0.1427	0.1780
Unaffected	113,045	0.99	-0.0044	0.1668

5.1.2 USE OF AFP IN SCREENING FOR AUTOSOMAL TRISOMIES

Independent estimates of the risk of Down's syndrome or an autosomal trisomy pregnancy can be obtained from (a) the maternal serum AFP result and (b) the *a priori* maternal age risk. Maternal serum AFP screening programmes can therefore be adapted to provide a combined risk of either Down's syndrome or of an autosomal trisomy.

5.1.2.1 RISK ESTIMATIONS FROM AFP LEVELS

It has been shown in Section 5.1.1.2 that the distributions of maternal serum AFP in the control samples, the Down's syndrome samples and the entire group of autosomal trisomy pregnancies conform to log Gaussian Distributions. The risk (likelihood ratio) that a particular AFP result is associated with a pregnancy with Down's syndrome or an autosomal trisomy can be estimated from the ratio of the heights of the distributions at that AFP level, as described in Section 4.2.8., using the means and standard deviations as given in Table 5-2.

5.1.2.2 MATERNAL AGE RISKS

The maternal age-specific risks used in this study (Table 5-3) are derived from those presented by Cuckle *et al* (1987), who combined the results of eight large published surveys of Down's syndrome in live births which specify the risk for single years of age. The

Table 5 - 3

Estimated maternal age-specific risk estimates of a Down's syndrome pregnancy or autosomal trisomy at birth and at mid-trimester.

Maternal age (years)	Risk of a Down's syndrome birth (Cuckle *et al*,1987)	Risk of an autosomal trisomy birth	Risk of a Down's syndrome pregnancy at mid-trimester	Risk of an autosomal trisomy pregnancy at mid-trimester
16	1:1572	1:1388	1:1257	1:1055
17	1:1565	1;1382	1:1252	1:1050
18	1:1556	1:1374	1:1245	1:1044
19	1:1544	1:1364	1:1235	1:1036
20	1:1528	1:1349	1:1222	1:1025
21	1:1507	1:1330	1:1206	1:1011
22	1:1481	1:1307	1:1185	1: 993
23	1:1447	1:1277	1:1158	1: 971
24	1:1404	1:1240	1:1123	1: 942
25	1:1351	1:1193	1:1081	1: 907
26	1:1286	1:1135	1:1029	1: 863
27	1:1208	1:1066	1: 996	1: 810
28	1:1119	1: 988	1: 895	1: 751
29	1:1018	1: 899	1: 814	1: 683
30	1: 909	1: 803	1: 727	1: 610
31	1: 796	1: 703	1: 637	1: 534
32	1: 683	1: 603	1: 546	1: 458
33	1: 574	1: 507	1: 459	1: 385
34	1: 474	1: 418	1: 379	1: 318
35	1: 384	1: 339	1: 307	1: 258
36	1: 307	1: 271	1: 246	1: 206
37	1: 242	1: 214	1: 194	1: 163
38	1: 189	1: 167	1: 151	1: 127
39	1: 146	1: 129	1: 117	1: 98
40	1: 112	1: 99	1: 90	1: 75
41	1: 85	1: 75	1: 68	1: 57
42	1: 65	1: 57	1: 52	1: 43
43	1: 49	1: 43	1: 39	1: 33
44	1: 37	1: 33	1: 30	1: 25
45	1: 28	1: 25	1: 22	1: 19

odds of an individual woman having a Down's syndrome fetus identified at the time of screening rather than an affected birth were calculated by multiplying the right-hand side of the odds ratio by 0.8. This factor was suggested by Cuckle *et al* (1987) and derived by comparing the estimated age-specific risk of Down's syndrome in the second trimester of pregnancy with the corresponding age-specific risk of Down's syndrome at birth. Because of the relatively small number of cases of trisomies 18 and 13 recorded in the literature, individual age-specific risks are not available for these chromosomal abnormalities. Therefore, in order to obtain specific age-related risks for an autosomal trisomy, Down's syndrome risks were multiplied by 1.1327. This multiplication factor was derived from the sum of known incidences of Down's syndrome per 1,000 births (Hook and Lindsjo, 1978), and trisomies 18 and 13 (Hook and Hamerton, 1977) divided by the incidence for Down's syndrome (1.324 + 0.123 + 0.0527 / 1.324 = 1.1327). The risks birth risks for an autosomal trisomy can be modified to reflect the odds of a fetus at the time of screening rather than at birth by multiplying the right-hand side of the odds ratio by 0.76. This factor was derived by comparing the collective age-specific risk of trisomies 21, 18 and 13 in the second trimester of pregnancy (Ferguson Smith and Yates, 1984) with the corresponding age-specific risk of an autosomal trisomy at birth. In the estimation of this factor, the age-specific risk of an autosomal trisomy was corrected to take into account the expected proportion (27%) of pregnancies complicated with anterior abdominal wall defect and/or neural tube defect in the second trimester of pregnancy (Linenbaum *et al*, 1987)

5.1.2.3 CALCULATION OF A COMBINED RISK FOR AN INDIVIDUAL PREGNANCY

A combined risk for an individual pregnancy can be obtained by multiplying the left-hand side of the maternal age-specific risk by the likelihood ratio. Mid-trimester risks were calculated for both Down's syndrome alone (Table 5-4) and for an autosomal trisomy (Table 5-5), using, for Down's syndrome, mid-trimester risks of a Down's syndrome pregnancy and likelihood ratios from the Down's syndrome log Gaussian distribution, and for autosomal trisomies, mid-trimester risks of an autosomal trisomy pregnancy and likelihood ratios from the autosomal trisomy distribution. For example, as shown in Figure 4a, a woman aged 31 years has an *a priori* mid trimester risk of an autosomal trisomy of 1:534. With an AFP level of 0.5 MOM the derived likelihood ratio for an autosomal trisomy is 3.07, which would modify her age-related risk to 1:170 (i.e. 3.07:534). On the other hand, a woman aged 37 years with an AFP result of 1.5 MOM (Figure 4b) has a likelihood ratio of an autosomal trisomy of 0.34 and an *a priori* risk of 1:163 from her age. This would give a combined mid trimester risk of an autosomal trisomy of 1:480 (i.e. 0.34:163).

Figures 5-5 and 5-6 show the change in mid-trimester risks for Down's syndrome alone and autosomal trisomies at different AFP levels (in MOM) and maternal ages. At any given AFP level the risks are lower for Down's syndrome alone than for autosomal trisomies. For example, an AFP level of 0.5 MOM generates a mid-trimester risk of an autosomal trisomy of 1:200 at maternal age 30, but a mid-trimester Down's syndrome risk of 1:230. At an AFP level of 1.5 MOM the mid-trimester risk of an autosomal

Table 5 - 4

Risk estimates of finding a Down's syndrome fetus at mid-trimester, based on maternal age and maternal serum alphafetoprotein (AFP) at 16-20 weeks gestation (MOM = multiples of the median).

Mat. Age (yrs)	AFP(MOM)										
	0.35	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85
25	1: 170	1: 220	1: 280	1: 350	1: 420	1: 510	1: 600	1: 700	1: 820	1: 940	1:1100
26	1: 160	1: 210	1: 270	1: 330	1: 400	1: 480	1: 570	1: 670	1: 780	1: 890	1:1000
27	1: 160	1: 210	1: 260	1: 320	1: 390	1: 470	1: 550	1: 650	1: 750	1: 870	1: 990
28	1: 140	1: 180	1: 230	1: 290	1: 350	1: 420	1: 500	1: 580	1: 680	1: 780	1: 890
29	1: 130	1: 170	1: 210	1: 260	1: 320	1: 380	1: 450	1: 530	1: 620	1: 710	1: 810
30	1: 120	1: 150	1: 190	1: 230	1: 290	1: 340	1: 400	1: 470	1: 550	1: 630	1: 720
31	1: 100	1: 130	1: 170	1: 210	1: 250	1: 300	1: 350	1: 410	1: 480	1: 550	1: 630
32	1: 87	1: 110	1: 140	1: 180	1: 210	1: 260	1: 300	1: 350	1: 410	1: 470	1: 540
33	1: 73	1: 95	1: 120	1: 150	1: 180	1: 220	1: 260	1: 300	1: 350	1: 400	1: 450
34	1: 61	1: 78	1: 100	1: 120	1: 150	1: 180	1: 210	1: 250	1: 290	1: 330	1: 380
35	1: 49	1: 63	1: 80	1: 100	1: 120	1: 140	1: 170	1: 200	1: 230	1: 270	1: 300
36	1: 39	1: 51	1: 64	1: 79	1: 100	1: 120	1: 140	1: 160	1: 190	1: 210	1: 240
37	1: 31	1: 40	1: 51	1: 63	1: 76	1: 91	1: 110	1: 130	1: 150	1: 170	1: 190
38	1: 24	1: 31	1: 39	1: 49	1: 59	1: 71	1: 84	1: 100	1: 110	1: 130	1: 150
39	1: 19	1: 24	1: 30	1: 38	1: 46	1: 55	1: 65	1: 76	1: 89	1: 100	1: 120
40	1: 14	1: 19	1: 23	1: 29	1: 35	1: 42	1: 50	1: 58	1: 68	1: 78	1: 89
41	1: 11	1: 14	1: 18	1: 22	1: 27	1: 32	1: 38	1: 44	1: 52	1: 59	1: 67
42	1: 8	1: 11	1: 14	1: 17	1: 20	1: 24	1: 29	1: 34	1: 39	1: 45	1: 51
43	1: 6	1: 8	1: 10	1: 13	1: 15	1: 18	1: 22	1: 25	1: 30	1: 34	1: 39
44	1: 5	1: 6	1: 8	1: 10	1: 12	1: 14	1: 17	1: 19	1: 23	1: 26	1: 30
45	1: 4	1: 5	1: 6	1: 7	1: 9	1: 10	1: 12	1: 14	1: 17	1: 19	1: 22

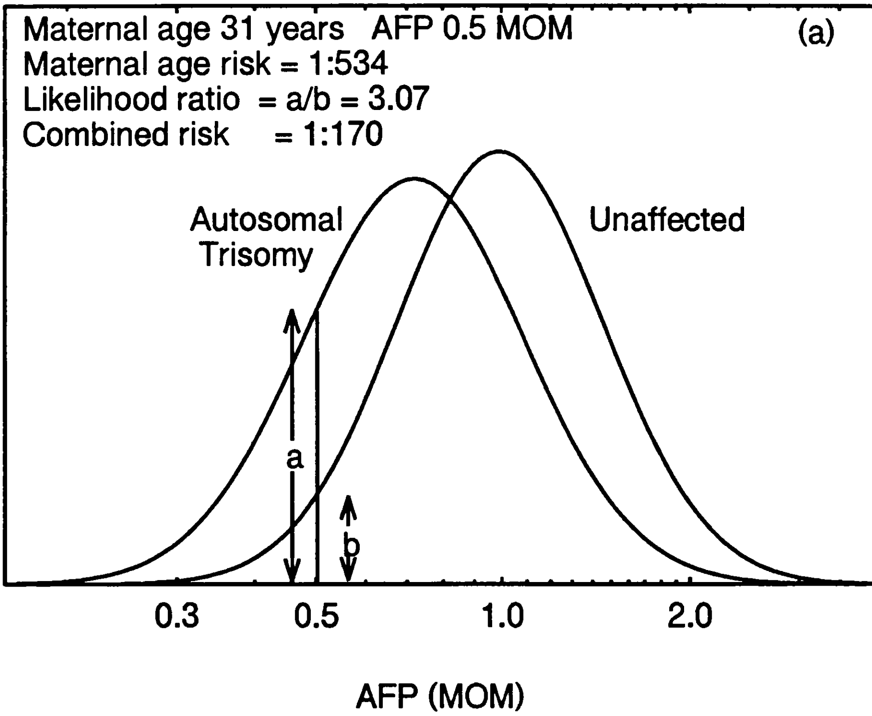
Mat. Age (yrs)	AFP(MOM)									
	0.90	0.95	1.00	1.10	1.20	1.30	1.40	1.50	1.80	2.00
25	1:1200	1:1400	1:1500	1:1800	1:2400	1:2800	1:3400	1:4000	1:6400	1:8300
26	1:1200	1:1300	1:1500	1:1800	1:2200	1:2700	1:3200	1:3800	1:6100	1:7900
27	1:1100	1:1300	1:1400	1:1800	1:2200	1:2600	1:3100	1:3700	1:5900	1:7700
28	1:1000	1:1200	1:1300	1:1600	1:2000	1:2400	1:2800	1:3300	1:5300	1:6900
29	1: 910	1:1000	1:1200	1:1500	1:1800	1:2100	1:2500	1:3000	1:4800	1:6300
30	1: 820	1: 930	1:1000	1:1300	1:1600	1:1900	1:2300	1:2700	1:4300	1:5400
31	1: 720	1: 820	1: 910	1:1100	1:1400	1:1700	1:2000	1:2400	1:3800	1:4900
32	1: 610	1: 700	1: 780	1: 980	1:1200	1:1400	1:1700	1:2000	1:3200	1:4200
33	1: 520	1: 590	1: 660	1: 820	1:1000	1:1200	1:1400	1:1700	1:2700	1:3600
34	1: 430	1: 490	1: 540	1: 680	1: 820	1:1000	1:1200	1:1400	1:2200	1:2900
35	1: 340	1: 380	1: 440	1: 550	1: 670	1: 810	1: 960	1:1100	1:1800	1:2400
36	1: 280	1: 320	1: 350	1: 440	1: 530	1: 650	1: 770	1: 910	1:1500	1:1900
37	1: 220	1: 250	1: 280	1: 350	1: 420	1: 510	1: 610	1: 720	1:1100	1:1500
38	1: 170	1: 190	1: 220	1: 270	1: 330	1: 400	1: 470	1: 560	1: 890	1:1200
39	1: 130	1: 150	1: 170	1: 210	1: 250	1: 310	1: 370	1: 430	1: 690	1: 900
40	1: 100	1: 120	1: 130	1: 160	1: 200	1: 240	1: 280	1: 330	1: 530	1: 690
41	1: 76	1: 87	1: 100	1: 120	1: 150	1: 180	1: 210	1: 250	1: 400	1: 520
42	1: 58	1: 67	1: 74	1: 93	1: 110	1: 140	1: 160	1: 190	1: 310	1: 400
43	1: 44	1: 50	1: 56	1: 70	1: 85	1: 100	1: 120	1: 140	1: 230	1: 300
44	1: 34	1: 38	1: 43	1: 54	1: 65	1: 79	1: 94	1: 110	1: 180	1: 230
45	1: 25	1: 28	1: 31	1: 39	1: 48	1: 58	1: 69	1: 81	1: 130	1: 170

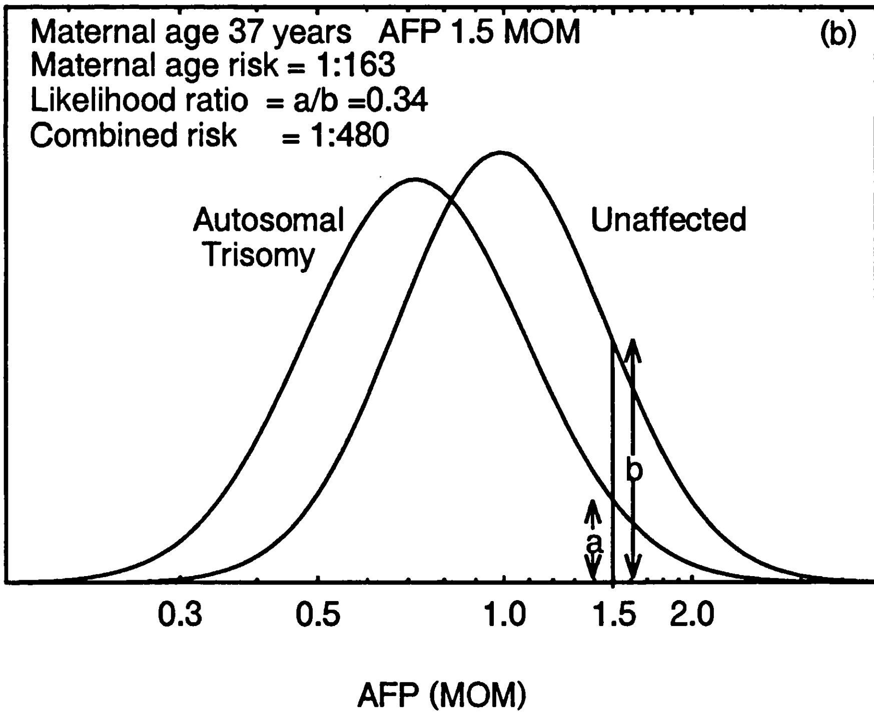
Table 5 - 5

Risk estimates of finding an autosomal trisomy fetus at mid-trimester, based on maternal age and maternal serum alphafetoprotein (AFP) at 16-20 weeks gestation (MOM = multiples of the median).

Mat. Age (yrs)	AFP(MOM) 0.35	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85
25	1: 120	1: 170	1: 230	1: 300	1: 370	1: 460	1: 550	1: 650	1: 750	1: 860	1: 980
26	1: 110	1: 160	1: 220	1: 280	1: 360	1: 430	1: 520	1: 620	1: 710	1: 810	1: 930
27	1: 100	1: 150	1: 200	1: 260	1: 330	1: 410	1: 490	1: 580	1: 670	1: 760	1: 870
28	1: 94	1: 140	1: 190	1: 240	1: 310	1: 380	1: 460	1: 540	1: 620	1: 710	1: 810
29	1: 88	1: 130	1: 170	1: 220	1: 280	1: 340	1: 410	1: 490	1: 560	1: 640	1: 730
30	1: 79	1: 110	1: 150	1: 200	1: 250	1: 310	1: 370	1: 440	1: 500	1: 580	1: 660
31	1: 69	1: 100	1: 130	1: 170	1: 220	1: 270	1: 320	1: 380	1: 440	1: 500	1: 570
32	1: 59	1: 85	1: 110	1: 150	1: 190	1: 230	1: 280	1: 330	1: 380	1: 430	1: 490
33	1: 50	1: 71	1: 100	1: 130	1: 160	1: 190	1: 230	1: 280	1: 320	1: 360	1: 410
34	1: 41	1: 59	1: 80	1: 100	1: 130	1: 160	1: 190	1: 230	1: 260	1: 300	1: 340
35	1: 33	1: 48	1: 65	1: 84	1: 110	1: 130	1: 160	1: 180	1: 210	1: 240	1: 280
36	1: 27	1: 38	1: 52	1: 67	1: 85	1: 100	1: 120	1: 150	1: 170	1: 190	1: 220
37	1: 21	1: 30	1: 41	1: 53	1: 67	1: 82	1: 100	1: 120	1: 130	1: 150	1: 180
38	1: 16	1: 23	1: 32	1: 41	1: 52	1: 64	1: 77	1: 91	1: 100	1: 120	1: 140
39	1: 13	1: 18	1: 25	1: 32	1: 40	1: 49	1: 59	1: 70	1: 81	1: 92	1: 110
40	1: 10	1: 14	1: 19	1: 24	1: 31	1: 38	1: 45	1: 54	1: 62	1: 71	1: 81
41	1: 7	1: 11	1: 14	1: 19	1: 23	1: 29	1: 35	1: 41	1: 47	1: 54	1: 61
42	1: 6	1: 8	1: 11	1: 14	1: 18	1: 22	1: 26	1: 31	1: 36	1: 41	1: 46
43	1: 4	1: 6	1: 8	1: 11	1: 14	1: 17	1: 20	1: 24	1: 27	1: 31	1: 35
44	1: 3	1: 5	1: 6	1: 8	1: 10	1: 13	1: 15	1: 18	1: 21	1: 24	1: 27
45	1: 2	1: 4	1: 5	1: 6	1: 8	1: 10	1: 12	1: 14	1: 16	1: 18	1: 20

Mat. Age (yrs)	AFP(MOM) 0.90	0.95	1.00	1.10	1.20	1.30	1.40	1.50	1.80	2.00
25	1:1100	1:1200	1:1300	1:1600	1:1900	1:2100	1:2400	1:2700	1:3500	1:4100
26	1:1000	1:1200	1:1300	1:1500	1:1800	1:2000	1:2300	1:2500	1:3300	1:3900
27	1: 980	1:1100	1:1200	1:1400	1:1700	1:1900	1:2100	1:2600	1:3100	1:3700
28	1: 900	1:1000	1:1100	1:1300	1:1500	1:1700	1:2000	1:2200	1:2900	1:3400
29	1: 820	1: 910	1:1000	1:1200	1:1400	1:1600	1:1800	1:2000	1:2600	1:3100
30	1: 730	1: 810	1: 900	1:1100	1:1200	1:1400	1:1600	1:1800	1:2300	1:2800
31	1: 640	1: 710	1: 790	1: 940	1:1100	1:1200	1:1400	1:1600	1:2100	1:2400
32	1: 550	1: 610	1: 670	1: 800	1: 930	1:1100	1:1200	1:1300	1:1800	1:2100
33	1: 460	1: 510	1: 570	1: 680	1: 790	1: 900	1:1000	1:1100	1:1500	1:1800
34	1: 380	1: 420	1: 470	1: 560	1: 650	1: 740	1: 840	1: 940	1:1200	1:1400
35	1: 310	1: 430	1: 380	1: 450	1: 530	1: 600	1: 680	1: 760	1: 990	1:1200
36	1: 250	1: 270	1: 300	1: 360	1: 420	1: 480	1: 540	1: 610	1: 790	1: 940
37	1: 200	1: 220	1: 240	1: 290	1: 330	1: 380	1: 430	1: 480	1: 630	1: 470
38	1: 150	1: 170	1: 190	1: 220	1: 260	1: 300	1: 330	1: 370	1: 490	1: 580
39	1: 120	1: 130	1: 140	1: 170	1: 200	1: 230	1: 260	1: 290	1: 380	1: 450
40	1: 90	1: 100	1: 110	1: 130	1: 150	1: 170	1: 200	1: 220	1: 290	1: 340
41	1: 69	1: 76	1: 84	1: 100	1: 120	1: 130	1: 150	1: 170	1: 220	1: 260
42	1: 52	1: 57	1: 63	1: 75	1: 88	1: 100	1: 110	1: 130	1: 170	1: 200
43	1: 40	1: 44	1: 49	1: 58	1: 67	1: 77	1: 87	1: 97	1: 130	1: 150
44	1: 30	1: 33	1: 37	1: 44	1: 51	1: 58	1: 66	1: 74	1: 96	1: 110
45	1: 23	1: 25	1: 28	1: 33	1: 39	1: 44	1: 50	1: 56	1: 73	1: 86





<u>Figure 5 - 4</u>

Combining (a) maternal age risk at age 31 with the likelihood ratio from AFP level of 0.5 MOM or (b) maternal age at age 37 with the likelihood ratio from AFP level of 1.5 MOM, to give a combined risk of an autosomal trisomy.

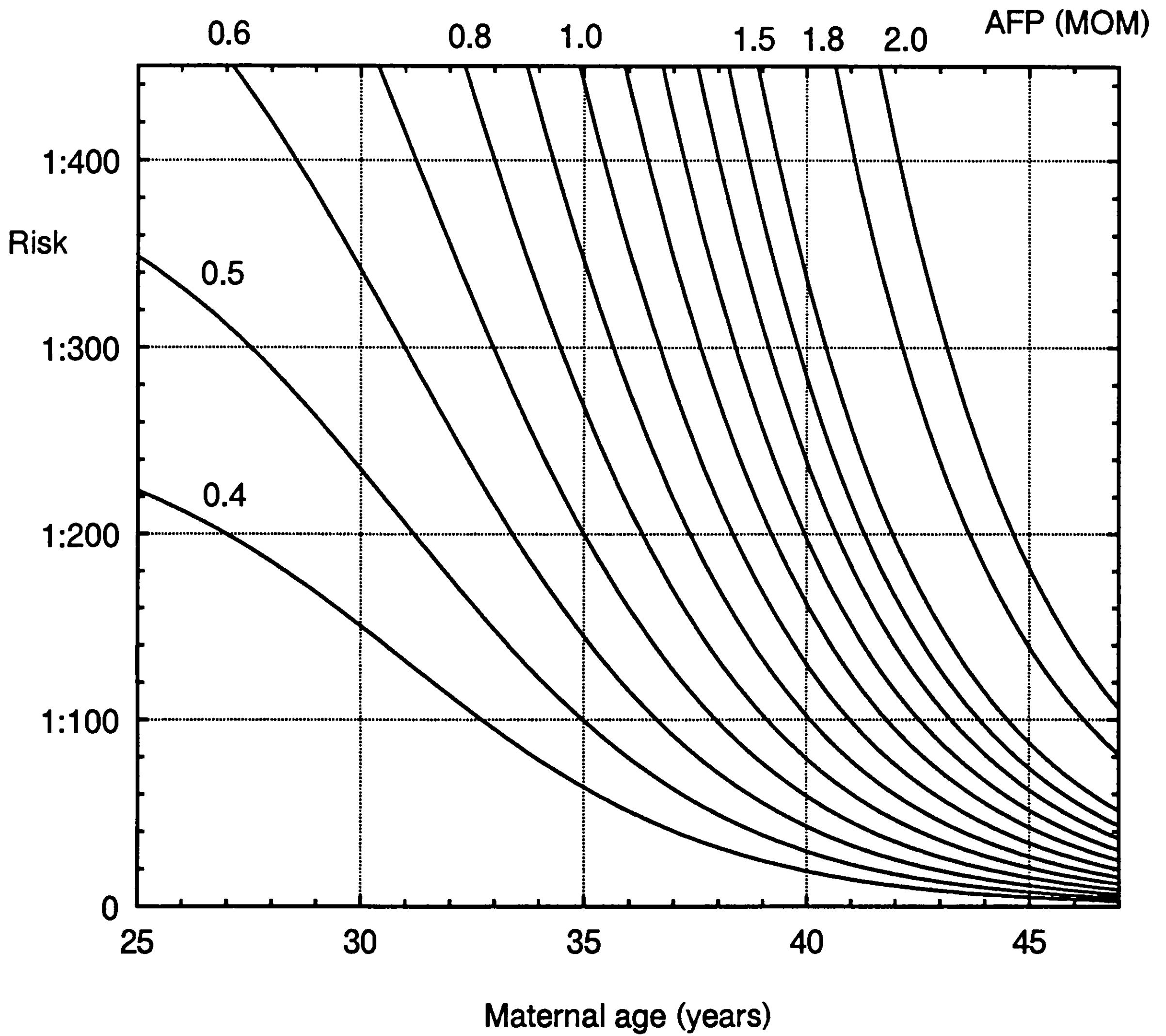


Figure 5 - 5

Risk of a Down's syndrome pregnancy at mid-trimester based on a combination of maternal age and maternal serum alphafetoprotein (AFP) level (MOM = multiple of the median).

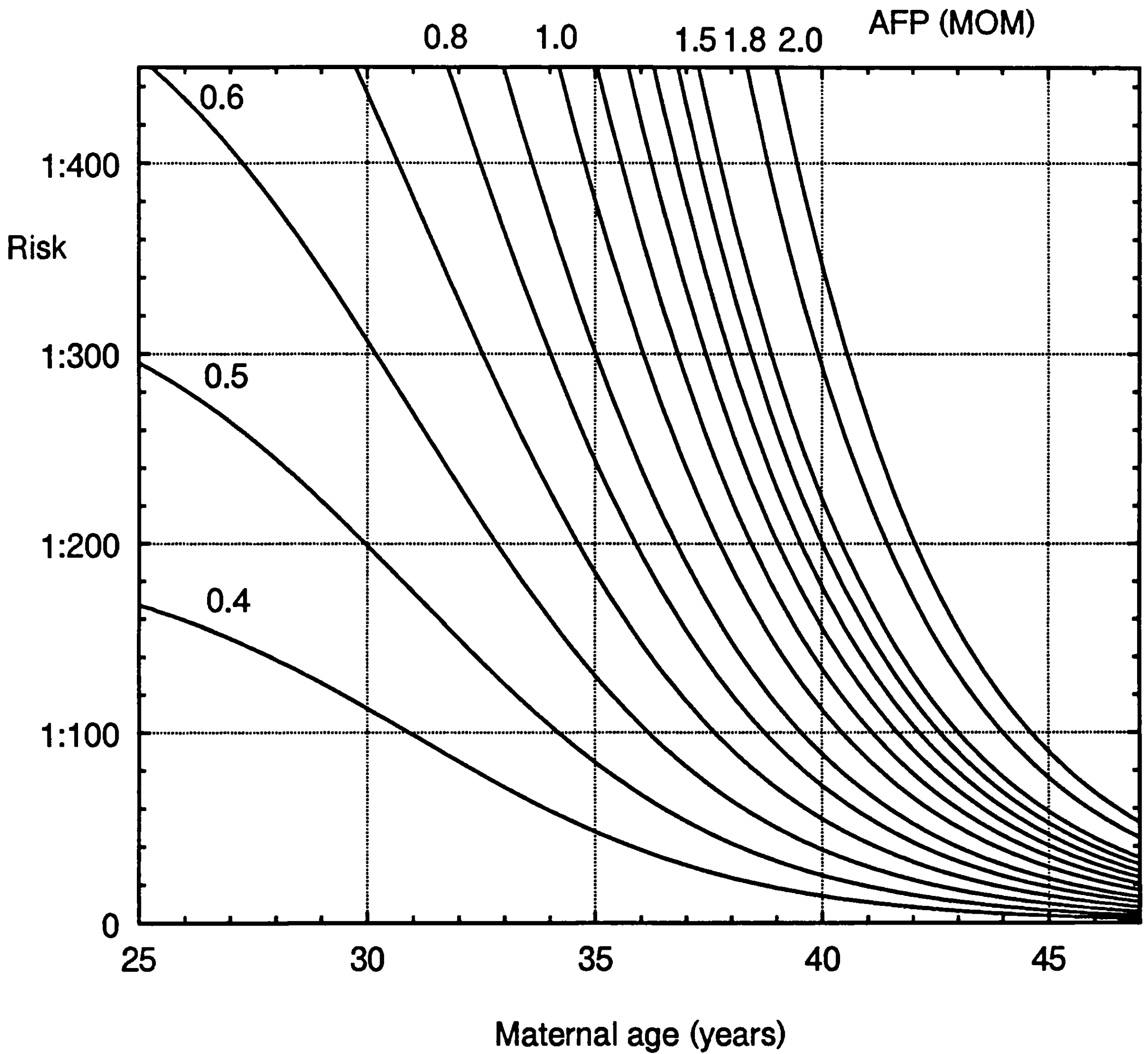


Figure 5 - 6

Risk of an autosomal trisomy pregnancy at mid-trimester based on a combination of maternal age and maternal serum alphafetoprotein (AFP) level (MOM = multiple of the median).

trisomy is 1:220 at age 40 but the mid-trimester risk of Down's syndrome is 1:330. This is mainly due to the population maternal age risk of an autosomal trisomy being higher than that for Down's syndrome alone, but the larger standard deviation of the autosomal trisomy distribution, causing greater likelihood ratios (Figure 5-7) is also a contributory factor.

5.1.2.4 PREDICTED DETECTION RATES

Detection and corresponding false positive rates were estimated in the west of Scotland pregnant population as described in Section 4.2.9. Table 5-6 shows a comparison of detection and false positive rates, at a range of mid trimester threshold risks, using either (a) the risks derived from the autosomal trisomy log Gaussian distribution and population mid-trimester autosomal trisomy risks, or (b) the risks derived from the Down's syndrome log Gaussian distribution and population mid-trimester Down's syndrome risks. It can be seen that at all cut-off risks shown the detection rate for Down's syndrome and the corresponding false positive rate are lower when method (b), using risks derived for Down's syndrome alone, are used. However at any given detection rate the false positive rates are equivalent. Using method (a), the overall detection rate for all three autosomal trisomies combined is slightly higher than for Down's syndrome alone. This is due to trisomy 18 pregnancies having slightly lower AFP values than Down's syndrome, and therefore having a slightly increased rate of detection.

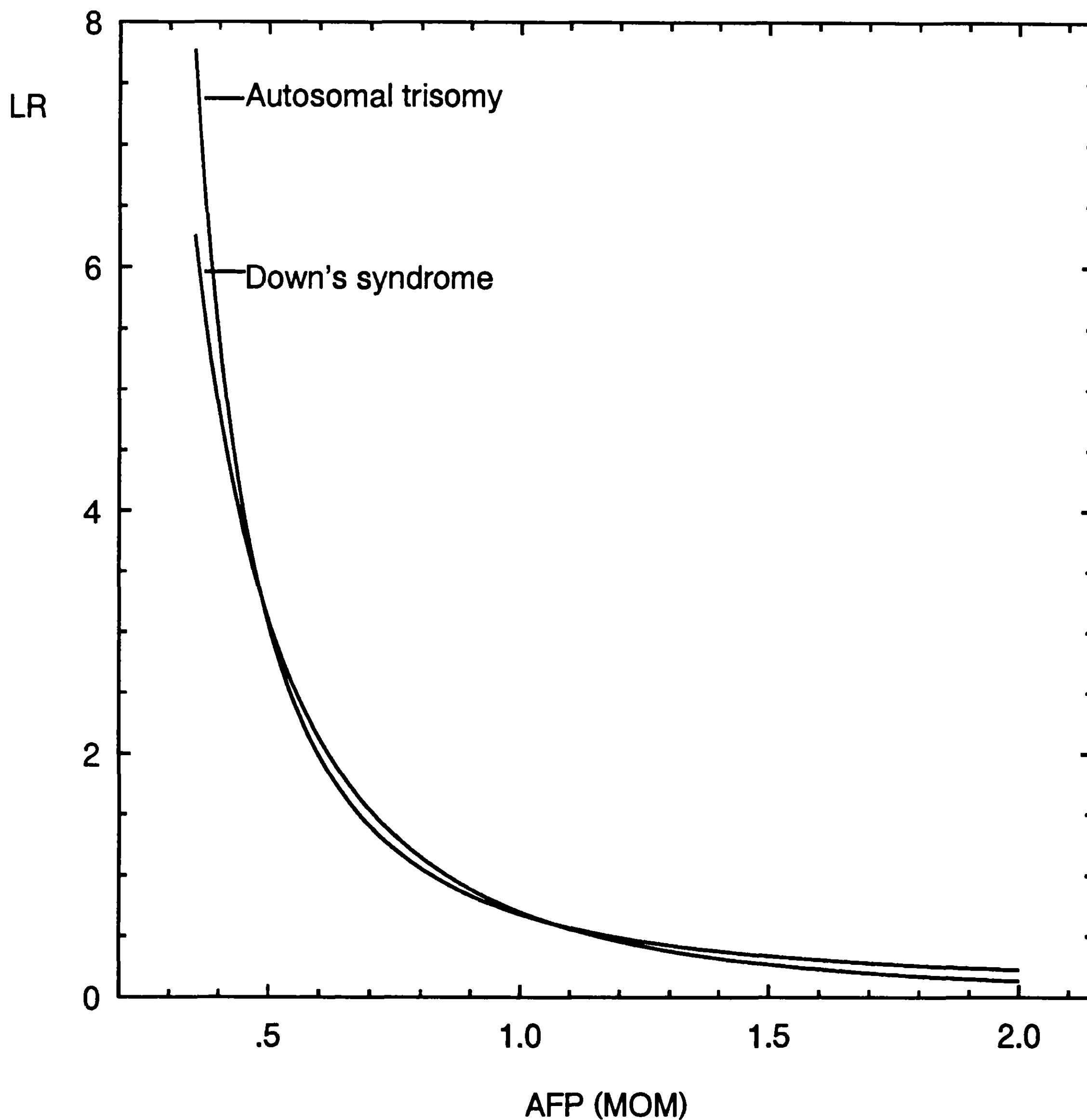


Figure 5 - 7

Likelihood ratios for autosomal trisomies and for Down's syndrome at alphafetoprotein (AFP) levels between 0.35 and 2.00 multiples of the median (MOM) calculated from the log Gaussian distributions with means and standard deviations given in Table 5-2.

Table 5 - 6

Comparison of detection and false positive rates at varying mid trimester risk thresholds in the west of Scotland pregnant population using (a) mid-trimester risks of an autosomal trisomy or (b) mid-trimester risks of Down's syndrome.

Cut-off risk	(a) Autosomal trisomy risks			(b) Down's risks	
	False +ve rate (%)	Det^n rate Aut.Tr. (%)	Det^n rate Down's (%)	False +ve rate (%)	Det^n rate Down's (%)
1:100	1.9	20	18	1.2	16
1:150	3.2	26	24	2.0	20
1:200	4.4	31	29	3.3	26
1:250	5.7	34	32	4.5	30
1:280	6.6	37	35	5.4	32
1:350	8.5	41	40	8.0	39

In practice, screening cannot distinguish between the different types of autosomal trisomy. Therefore the risks of an autosomal trisomy were considered more appropriate and have been used in the west of Scotland AFP/age prenatal screening programme for chromosome abnormalities. Using the above AFP and age parameters, an overall detection rate of 37% for autosomal trisomies was estimated at a false positive rate of 6.6%, using a threshold risk of 1:280. The corresponding figures using maternal age 35 years and over as the indicator for amniocentesis in the west of Scotland population, are 30% detection with a 6.7% false positive rate. The use of combined AFP/age screening extends the age range for screening to 25 years and over, but it should be noted that there is considerable variation in detection and false positive rates between different maternal age groups (Table 5-7). For example, only 12% of the autosomal trisomies in the age group 25-29 years are identified within the high risk group, compared with 71% in the 35-37 years group and 100% in the 40 years and over age group.

Combined AFP/age screening improves the overall odds of finding an affected pregnancy when compared with screening by maternal age ≥35 years. Using maternal age the overall odds are 1:125 and using AFP/age screening at a mid-trimester risk threshold of 1:280 the odds are (Table 5-8). Using AFP/age screening, for women aged 35 years and over, assigned to the high risk group, the overall odds of an affected pregnancy are 1:80, compared with 1:470 in the low risk (<1:280) group. For women age 25-34, the overall risk of an autosomal trisomy is 1:650. For the high risk (≥1:280) group on AFP/age screening the overall odds of an affected pregnancy are

Table 5 - 7

Comparison of false positive rates and detection rates for autosomal trisomies within different maternal age groups in the west of Scotland.

Age group (years)	% all pregnancies	% all autosomal trisomies	% in age group with risk ≥1:280	% autosomal trisomies in age group detected
<25	40	23	-	-
25-29	36	25	3	12
30-34	18	21	10	33
35-37	4.4	12	37	71
38-39	1.3	7	80	94
≥40	1.0	12	100	100
overall	100	100	6.6	37

Table 5 - 8

Comparison of overall odds of an autosomal trisomy pregnancy at mid-trimester

	OVERALL ODDS OF AN AFFECTED PREGNANCY
(a) POPULATION RISKS	
Overall	1:570
Age ≥35 years	1:125
Age 25-34 years	1:650
(b) WITH AGE/AFP RISK ≥1:280	
Overall	1:100
Age ≥35 years	1: 80
Age 25-34 years	1:165
(c) WITH AGE /AFP RISK <1:280	
Overall	1:840
Age ≥35 years	1:470
Age 25-34 years	1:780

1:165, compared with 1:780 if in the low risk (<1:280) group. Screening for autosomal trisomies using AFP/age therefore gives women aged 25 years and over who are assigned to the low risk group a reduced overall odds of having an affected pregnancy compared with their overall *a priori* odds.

5.1.3 ROUTINE PROSPECTIVE SCREENING FOR AUTOSOMAL TRISOMIES USING AFP/AGE

Screening for autosomal trisomies using AFP/age was added to the existing west of Scotland screening programme for neural tube defects in July 1987. AFP results were converted to MOM, using the appropriate gestation and an individual woman's combined risk of an autosomal trisomy derived from Table 5-5. Where maternal weight was available MOM values were corrected for maternal weight by the formula

$$MOM^* = MOM \times 0.4592 \times (1.01272)^{WT}$$

where MOM* is the weight adjusted AFP value in MOM and WT is the maternal weight in kilogrammes (Wald *et al.*, 1981). A threshold risk of 1:280 was used to define the high risk group.

During the period July 1987 to December 1990 there were 128,824 pregnancies and 100,481 women chose to have prenatal screening, an uptake of 78%. The results of screening during this period are summarised in Tables 5-9 and 5-10. The false positive rate, after some gestations had been reassessed, was 6.1%, with 44% of these women being aged 35 years and over and the remaining 56% being aged between 25 and 34 years. The overall uptake of amniocentesis

Table 5 - 9

Prospective AFP/age screening for autospmal trisomies in the west of Scotland, July 1987 to December 1990: False positive rate and uptake of diagnostic testing.

Total no of pregnancies screened	:	100,481
High risk group (Risk ≥1:280)		
Number (%) screen positive	:	6,149 (6.1%)
≥35 years	:	2,706
<35 years	:	3,443
Uptake of amniocentesis ≥35 years	:	56%
<35 years	:	32%
Overall	:	42%
Low risk group (Risk <1:280, Age ≥35 years)		
Number of women	:	2,159
% of all pregnancies ≥35 years	:	44%
Proportion having diagnostic testing	:	23%

Table 5 - 10

Prospective AFP/age screening for autosomal trisomies in the west of Scotland, July 1987 to December 1990: Detection rates.

Down's syndrome in screened population	94
Trisomy 18 in screened population	13
Trisomy 13 in screened population	3
Total autosomal trisomy in screened population	110
Number (%) Down's syndrome with risk ≥1:280	39 (41%)
Number (%) trisomy 18 with risk ≥1:280	7 (54%)
Number (%) trisomy 13 with risk ≥1:280	1 (33%)
Number (%) all autosomal trisomy with risk ≥1:280	47 (43%)
Number (%) Down's syndrome prenatally diagnosed	22 (23%)
Number (%) trisomy 18 prenatally diagnosed	6 (46%)
Number (%) trisomy 18 prenatally diagnosed	0 (0%)
Number (%) all autosomal trisomies prenatally diagnosed	28 (25%)

by women in the high risk group was 42%, with a marked difference between the women age 35 years and over, who had an uptake of 56%, compared with those under 35 years, with an uptake of only 32%. The uptake of prenatal diagnosis in the 44% of women age 35 years and over who where assigned to the low risk group was 23%.

The overall detection rate for autosomal trisomies by screening was 43%, but the rate prenatally diagnosed was only 25% due mainly to the low uptake of amniocentesis by women aged less than 35 years. Of the 39 Down's syndrome pregnancies in the high risk group, 25 were in women aged 35 years and over, and of these 18 (72%) were prenatally diagnosed. The remaining 14 were in women aged 25-34 years and only 4 of these (29%) were prenatally diagnosed. The proportion of trisomy 18 pregnancies actually prenatally diagnosed (6 out of 7 assigned to the high risk group) was greater than that for Down's syndrome. This is due to trisomy 18 pregnancies tending to have slightly lower AFP results and hence higher risks than Down's syndrome pregnancies.

A summary of all the autosomal trisomy pregnancies identified cytogenetically relating to this period of screening is given in Table 5-11. There were 170 autosomal trisomy pregnancies in total (1.3/1000 pregnancies), consisting of 139 Down's syndrome (1.1/1000 pregnancies), 23 trisomy 18 and 8 trisomy 13. Of these, 59 (35%) were prenatally diagnosed, for a variety of indications: Risk ≥1:280 from AFP/age screening, maternal age 35 years or over, high maternal serum AFP (≥2.0 MOM) and abnormal ultrasound findings. The largest group (28, 47%) were those identified by AFP/age screening.

Table 5 - 11

Summary of all autosomal trisomy pregnancies in the west of Scotland for the period screened July 1987 to December 1990.

	Down's syndrome	Trisomy 18	Trisomy 13	Total autosomal trisomy
Singleton pregnancies				
Screened-HR-PND	22	6	0	28
Screened-HR-Born	17	1	1	19
Screened-LR-PND	2	0	0	2
Screened-LR-Born	53	6	2	61
High AFP-PND	1	1	1	3
1st Tri CVS-MA-PND	8	5	0	13
Amnio-MA-PND	7	1	2	10
CVS/Amnio U/S-PND	3	0	0	3
Not screened-Born	23	3	2	28
Twin pregnancies				
Screened-Born	2	0	0	2
Not screened-Born	1	0	0	1
Total	139	23	8	170

HR = High risk group (Risk ≥1:280)
LR = Low risk group (Risk <1:280)
PND = Prenatally diagnosed
CVS = Chorionic villus sampling
Amnio = Amniocentesis
MA = Maternal age ≥35 years
High AFP = Maternal serum AFP ≥2.0 MOM
U/S = Abnormal ultrasound findings

PHASE 2

5.2 RETROSPECTIVE ANALYSIS OF PREGNANCY MARKERS IN STORED MATERNAL SERUM

A panel of serum samples consisting of 118 chromosomally abnormal pregnancies and 410 controls was used in the analysis of four maternal serum markers: intact hCG, SP_1, UE3 and Frβ-hCG.

5.2.1 ANALYTE LEVELS

5.2.1.1 HUMAN CHORIONIC GONADOTROPHIN

5.2.1.1.1 hCG LEVELS IN UNAFFECTED PREGNANCIES

Four hundred and ten control sera taken between 15-20 weeks gestation were analysed for intact hCG as described in Section 4.1.3.1. These consisted of 11 samples at 15 weeks gestation, 184 at 16 weeks, 145 at 17 weeks, 44 at 18 weeks, 16 at 19 weeks and 10 at 20 weeks. Median hCG levels declined slightly with advancing gestation between 15 and 20 weeks. Medians, calculated by linear interpolation,were 25 IU/ml at 15 weeks, 23 IU/ml at 16 and 17 weeks, 20 IU/ml at 18 weeks and 19 IU/ml at 19 and 20 weeks.

5.2.1.1.2 hCG LEVELS IN DOWN'S SYNDROME

The levels of intact hCG (in MOM) found in 49 Down's syndrome pregnancies, plotted at the appropriate completed week of gestation, are shown on Figure 5-8. The median hCG value for the pregnancies affected by Down's syndrome was found to be

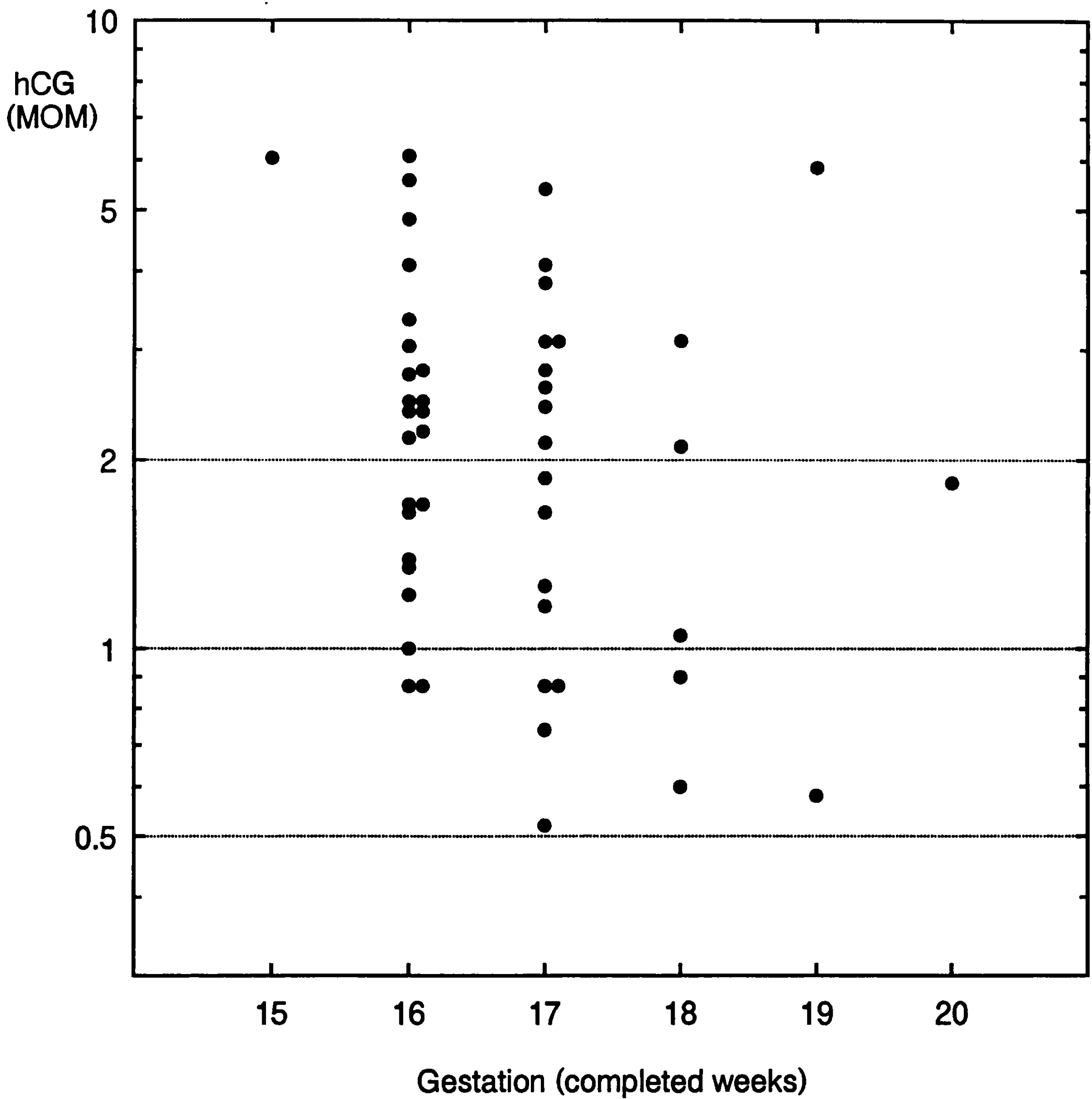


Figure 5 - 8

Maternal serum intact chorionic gonadotrophin (hCG) levels, in multiples of the median (MOM), in 49 Down's syndrome pregnancies compared with the levels in 410 unaffected controls.

significantly elevated at 2.18 MOM (95% CL 1.77 - 2.68 MOM) of the unaffected pregnancies ($p<0.001$ by t-test on unpaired variables). The proportions of control and Down's syndrome pregnancies with hCG levels equal to or greater than selected cut-off levels are shown in Table 5-12, with, for example 26 of the 49 Down's samples (53%) having levels greater than or equal to 2.0 MOM, compared with 35 of 410 controls (8.5%). A probability plot (Figure 5-9) shows that the distributions of hCG values in the control samples up to 2.0 MOM and in the Down's syndrome samples between 0.6 and 6.0 MOM fit log Gaussian distributions (Figure 5-10). The intersection point of the two curves is 1.59 MOM. The means and standard deviations of the distributions were estimated as described in Section 4.2.3.1 and are shown in Table 5-13.

5.2.1.1.3 hCG LEVELS IN OTHER CHROMOSOME ABNORMALITIES

The levels of hCG, in MOM, in sera from the other types of chromosomally abnormal pregnancies are shown in Figure 5-11 and their median values in Table 5-14. Differences between the distributions of the levels in the control samples and the different types of chromosome abnormality were determined by the Kolmorogov-Smirnov test (Table 5-14). There was no significant difference between the controls and the four trisomy 13 pregnancies, the four balanced translocations and the heterogeneous group of nine sex chromosome abnormalities. The group of eight unbalanced translocations appear to show some lowering of hCG levels ($0.05>p>0.01$), while the levels in the 12 trisomy 18 pregnancies were all below 0.6 MOM, with a median value

Table 5 - 12

Proportion of control, Down's syndrome and trisomy samples with maternal serum chorionic gonadotrophin (hCG) levels, in multiples of the median (MOM), greater than or equal to selected cut-off values.

hCG (MOM)	Controls (n=410)		Down's (n=49)		Trisomy 18 (n=12)	
	%	(n)	%	(n)	%	(n)
≥3.0	0.7	(3)	28.6	(14)	0	(0)
≥2.5	3.4	(14)	36.7	(18)	0	(0)
≥2.0	8.5	(35)	53.1	(26)	0	(0)
≥1.5	21.7	(89)	71.4	(35)	0	(0)
≥1.0	51.0	(209)	79.6	(39)	0	(0)
≥0.5	87.8	(360)	100	(49)	8.3	(1)

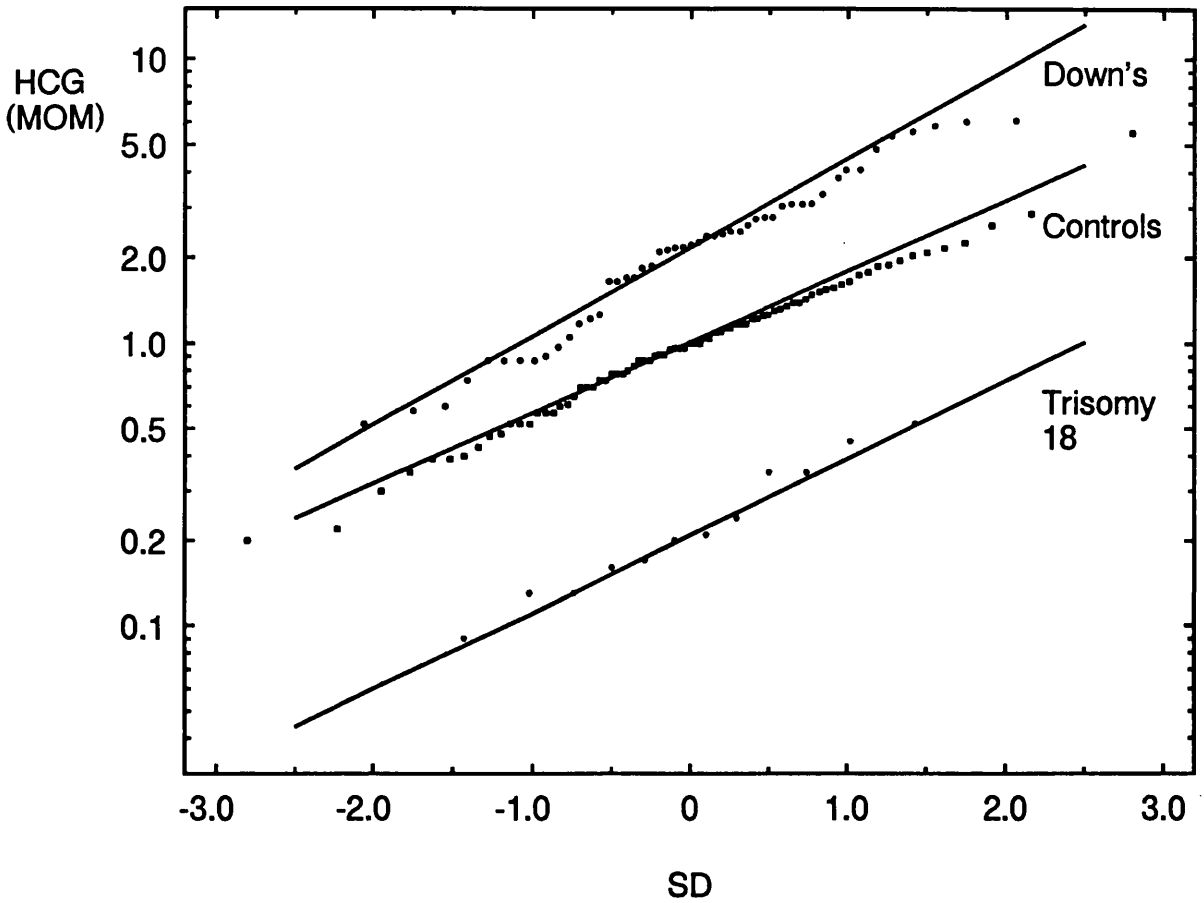


Figure 5 - 9

Probability plot of maternal serum chorionic gonadotrophin (hCG) levels, in multiples of the median (MOM), on a log scale, in selected unaffected control pregnancies, Down's syndrome pregnancies and trisomy 18 pregnancies. The continuous lines are those defined by log Gaussian distributions with means and standard deviations as given in Table 5-13.

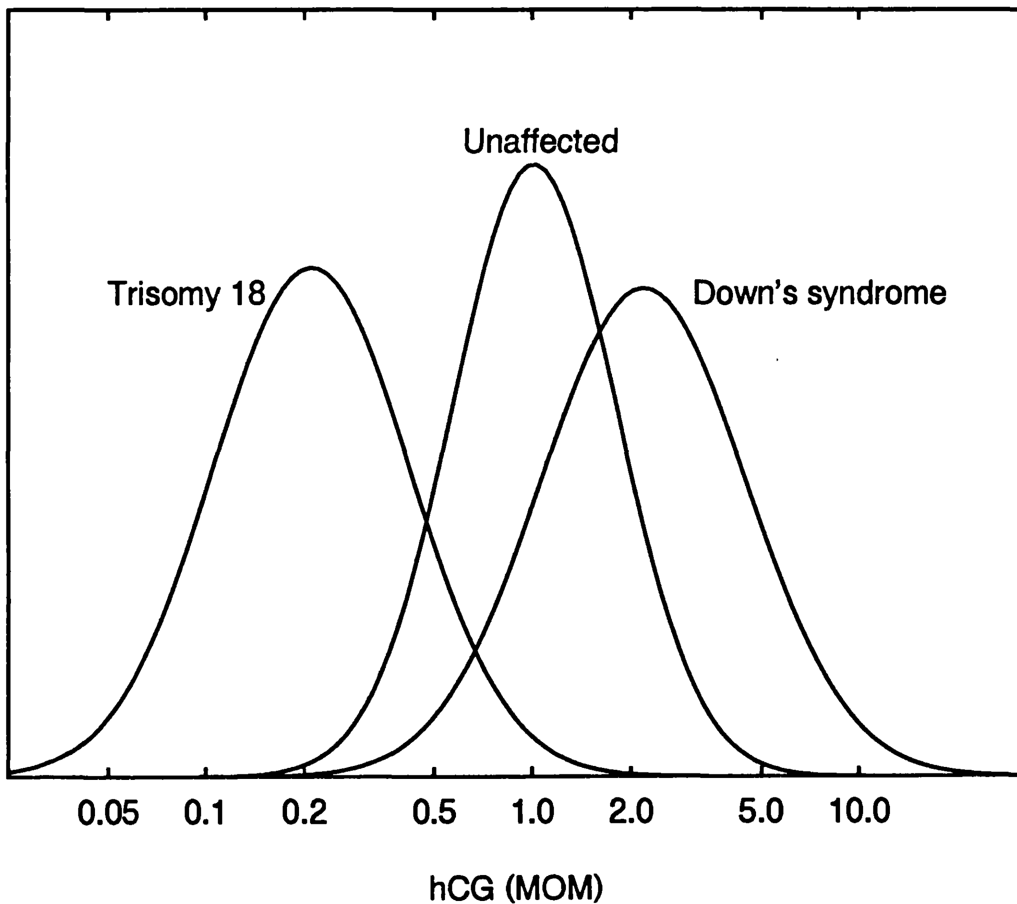


Figure 5 - 10

Log Gaussian distributions of maternal serum chorionic gonadotrophin (hCG) levels, in multiples of the median (MOM), in unaffected control pregnancies, Down's syndrome pregnancies and trisomy 18 pregnancies. These are plotted from the means and standard deviations of 410 unaffected, 49 Down's syndrome and 12 Trisomy 18 pregnancies as given in Table 5-13.

Table 5 - 13

Maternal serum chorionic gonadotrophin (hCG) medians, in multiples of the median (MOM), and means and standard deviations (SD) of the $\log_{10}$ distributions for the controls, Down's syndrome pregnancies and trisomy 18 pregnancies.

	n	Median (MOM)	Mean	SD
Controls	410	1.01	0.0043	0.2499
Down's	49	2.18	0.3385	0.3127
Trisomy 18	12	0.21	-0.6778	0.2730

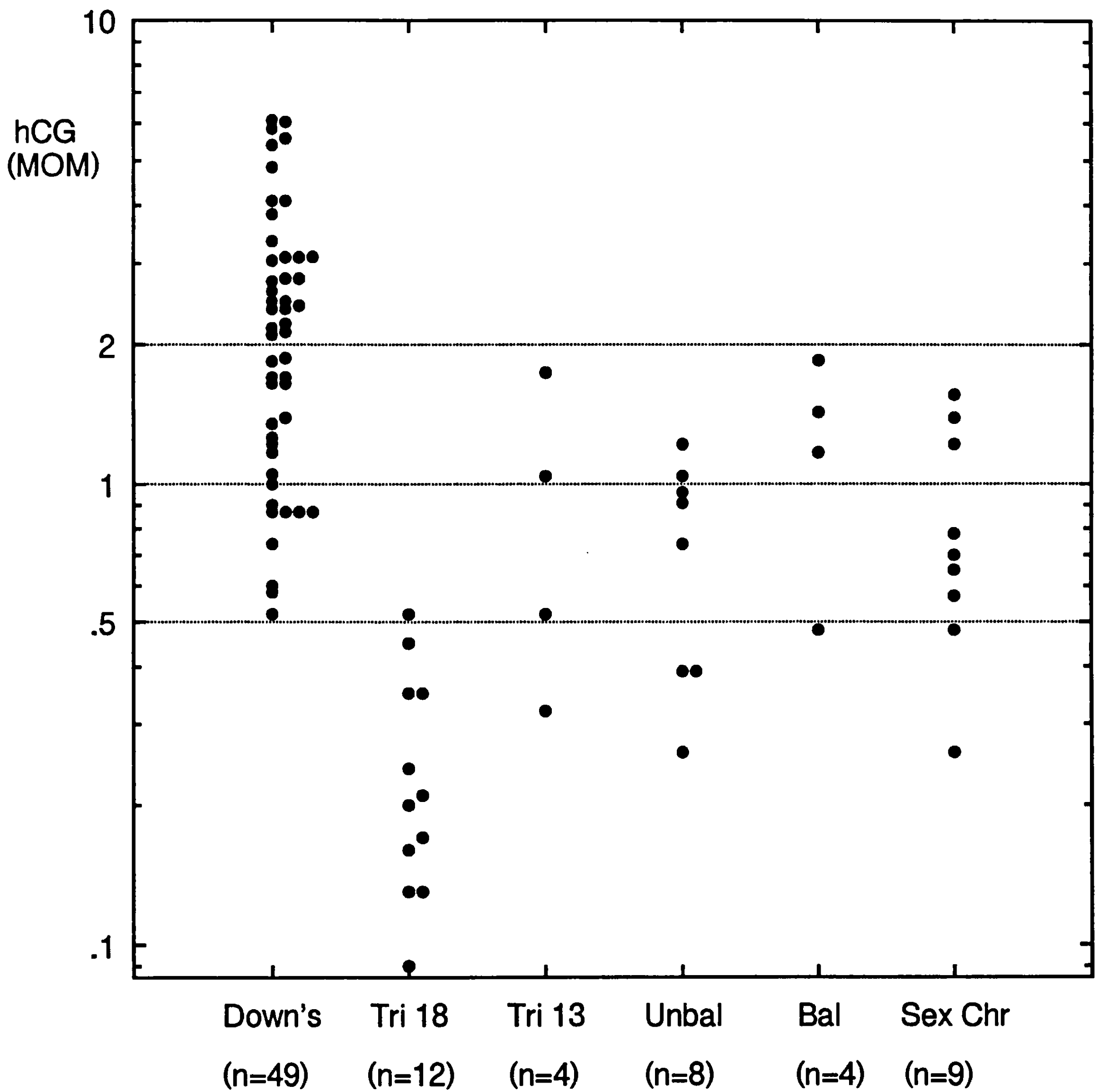


Figure 5 - 11

Maternal serum chorionic gonadotrophin (hCG) levels, in multiples of the median (MOM), in 86 pregnancies with chromosome abnormalities compared with the levels in 410 unaffected controls.

Table 5 - 14

Maternal serum chorionic gonadotrophin (hCG) median values, in multiples of the median (MOM), and significance testing by the Kolmogorov-Smirnov (K-S) test of the distributions of hCG levels in trisomy 18, trisomy 13, unbalanced translocations, balanced translocations and sex chromosome abnormalities against the control group. (D = absolute difference, p = probability).

	n	hCG median (MOM)	K-S test D	K-S test p
Trisomy 18	12	0.21	0.8757	<0.001
Trisomy 13	4	0.78	0.3749	>0.2
Unbalanced trans.	8	0.67	0.4745	0.021
Balanced trans.	4	1.39	0.3526	>0.2
Sex chrom. abn.	9	0.70	0.3367	>0.2

of 0.21 MOM, a highly significant difference ($p<0.001$).

Although the numbers are small, the trisomy 18 hCG values appear to fit a log Gaussian distribution (See probability plot, Figure 5-9). The log Gaussian distribution of hCG in the trisomy 18 samples, with mean and standard deviation as given in Table 5-13, appears to have a greater shift away from the levels in the controls than the Down's syndrome samples (Figure 5-10). The proportions of trisomy 18 samples with levels greater than or equal to selected cut-off levels are shown in Table 5-12, with only 1 case out of 12 (8.3%) having a value greater than or equal to 0.5 MOM. In comparison 360 out of 410 of the controls (87.8%) and all 49 (100%) of the Down's syndrome samples had hCG levels ≥0.5 MOM.

5.2.1.2 PREGNANCY SPECIFIC β-1 GLYCOPROTEIN

5.2.1.2.1 SP_1 LEVELS IN UNAFFECTED PREGNANCIES

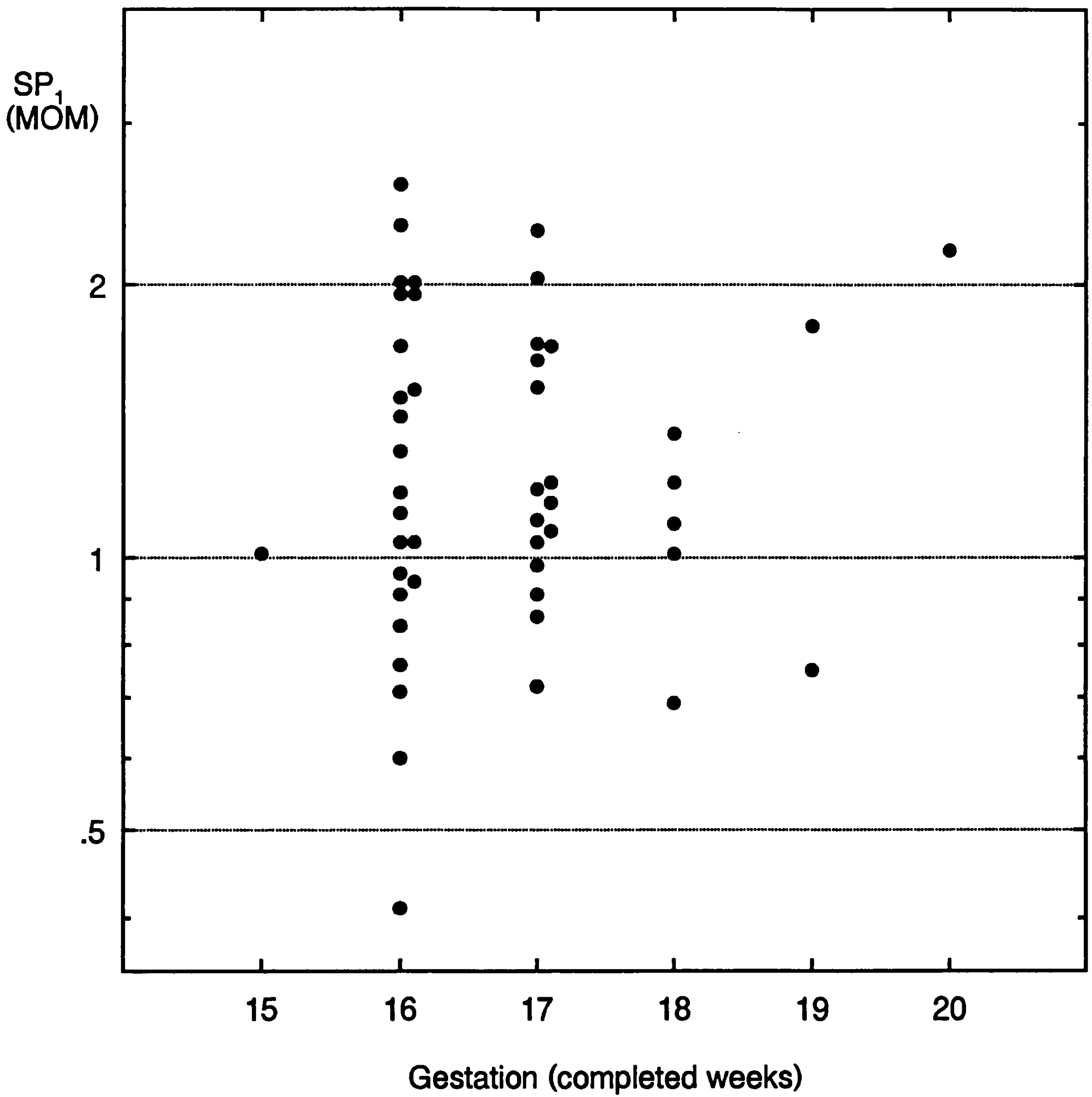
SP_1 was assayed as described in Section 4.1.4.1 in 377 controls at 15 to 20 weeks gestation, consisting of 11 samples at 15 weeks gestation, 179 at 16 weeks, 128 at 17 weeks, 39 at 18 weeks, 10 at 19 weeks and 10 at 20 weeks. Median SP_1 levels increased with advancing gestation between 15 and 20 weeks. The regressed median values in the controls were: 26.1 mg/L at 15 weeks, 28.2 mg/l at 16 weeks, 30.5 mg/l at 17 weeks, 32.9 mg/l at 18 weeks, 35.6 mg/l at 19 weeks and 38.5 mg/l at 20 weeks.

5.2.1.2.2 SP_1 LEVELS IN DOWN'S SYNDROME

The levels of SP_1, in MOM, in 48 Down's syndrome cases plotted at the appropriate completed week of gestation, are shown in Figure 5-12. The median SP_1 level in 48 Down's syndrome pregnancies was significantly elevated ($p<0.01$ by t-test on unpaired variables) at 1.17 MOM (95% CL 1.04 - 1.32 MOM) of the unaffected pregnancies. The proportions of control and Down's syndrome pregnancies with SP_1 levels equal to or greater than selected cut-off levels are shown in Table 5-15, with, for example 34 of the 48 Down's syndrome pregnancies (70.8%) having values greater than or equal to 1.0 MOM compared with 190 of 377 controls (50.4%). A probability plot (Figure 5-13) shows that the distributions of SP_1 values between 0.6 and 1.8 MOM in the controls and between 0.7 and 2.4 MOM in the Down's syndrome samples fit log Gaussian distributions (Figure 5-14). The intersection point of the two curves is 1.23 MOM. The means and standard deviations of the control and Down's syndrome SP_1 distributions were estimated as described in Section 4.2.3.1 and are shown in Table 5-16.

5.2.1.2.3 SP_1 LEVELS IN OTHER CHROMOSOME ABNORMALITIES

The levels of SP_1, in MOM, in the sera from the other chromosomally abnormal pregnancies are shown in Figure 5-15, and their median values in Table 5-17. Differences between the distributions of the SP_1 levels in the control samples and the other types of chromosome abnormality were assessed by the Kolmorgorov-Smirnov test (Table 5-17). The eight unbalanced



<u>Figure 5 - 12</u>

Maternal serum pregnancy specific ß-1 glycoprotein (SP_1) levels, in multiples of the median (MOM), in 48 Down's syndrome pregnancies compared with the levels in 377 unaffected controls.

Table 5 - 15

Proportion of control, Down' syndrome and trisomy 18 samples with maternal serum pregnancy specific ß-1 glycoprotein (SP_1) levels, in multiples of the median (MOM), greater than or equal to selected cut-off values.

SP_1 (MOM)	Controls (n=377)		Down's (n=48)		Trisomy 18 (n=9)	
	%	(n)	%	(n)	%	(n)
≥2.5	0	(0)	2.1	(1)	0	(0)
≥2.0	1.1	(14)	14.6	(7)	0	(0)
≥1.5	10.1	(38)	35.4	(17)	0	(0)
≥1.0	50.4	(190)	70.8	(34)	44.4	(4)
≥0.5	96.8	(365)	97.9	(47)	100	(9)

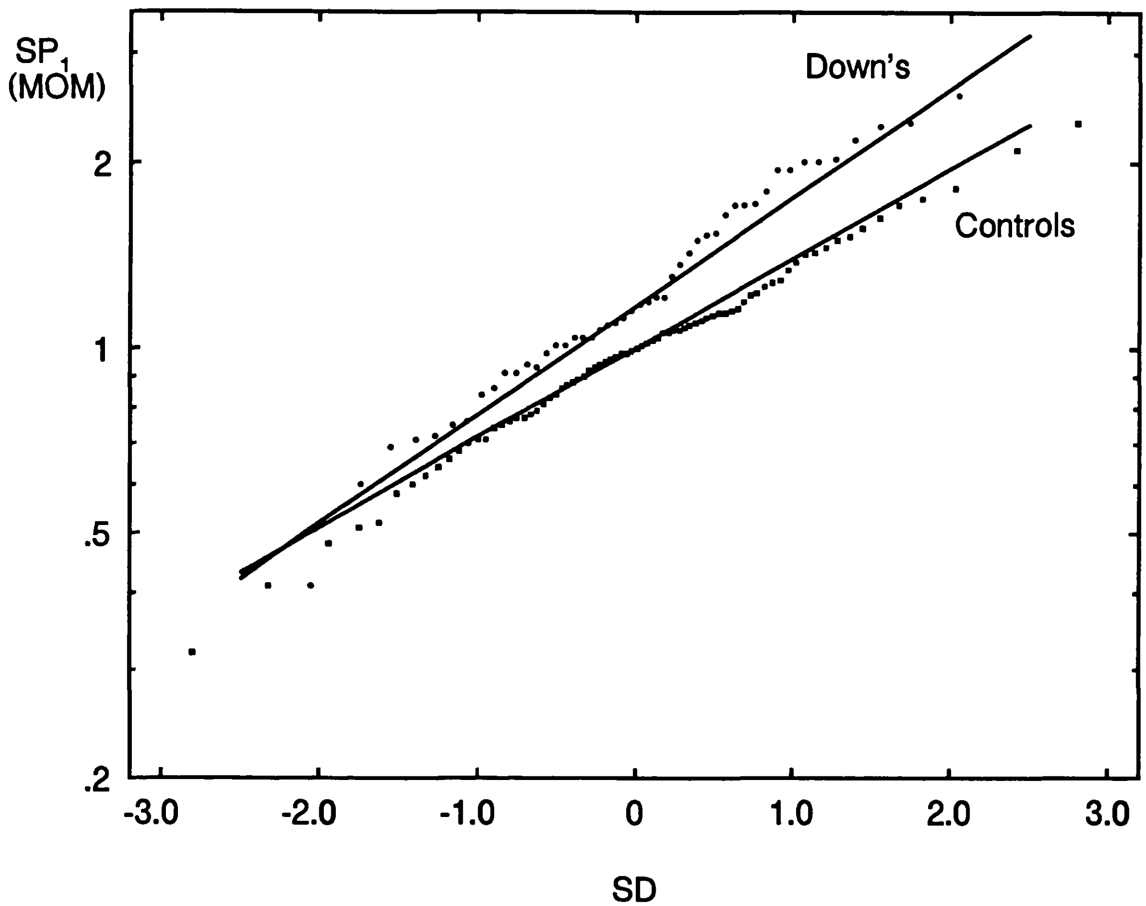


Figure 5 - 13

Probability plot of maternal serum pregnancy specific β-1 glycoprotein (SP_1) levels, in multiples of the median (MOM), on a log scale, in selected control pregnancies and those affected by Down's syndrome. The continuous lines are those defined by log Gaussian distributions with means and standard deviations as given in Table 5-16.

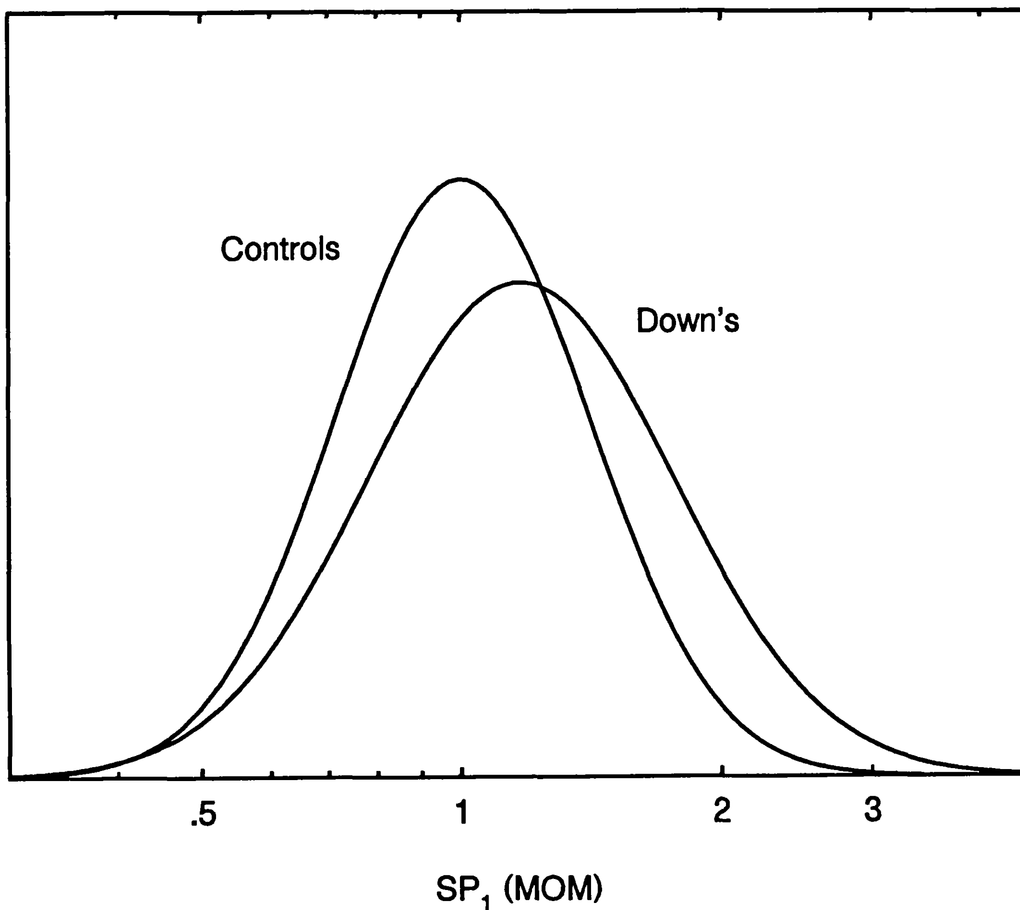


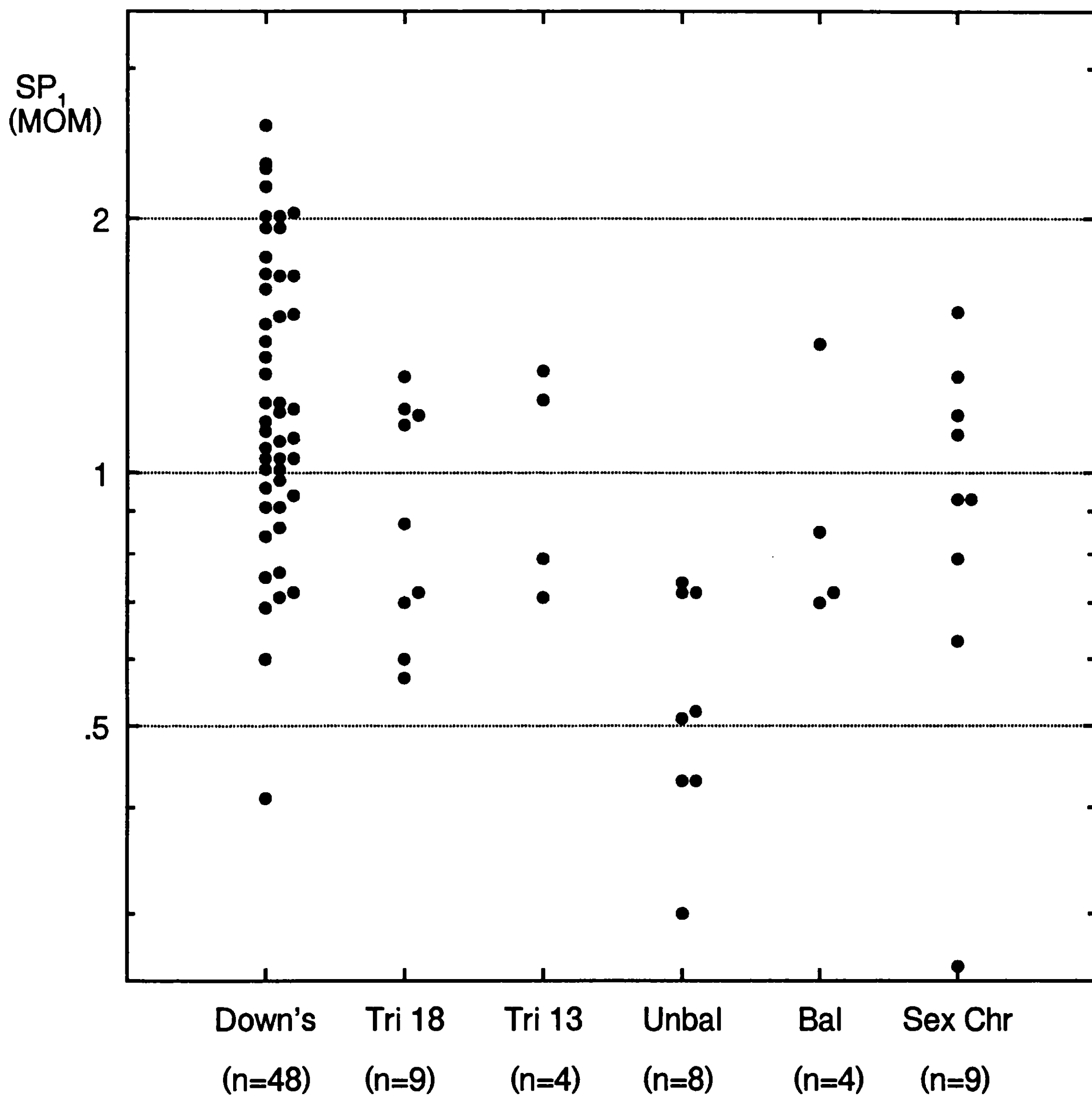
Figure 5 - 14

Log Gaussian distributions of maternal serum pregnancy specific β-1 glycoprotein (SP_1) levels, in multiples of the median (MOM), in controls and Down's syndrome pregnancies. These are plotted from the means and standard deviations of 377 unaffected and 48 Down's syndrome pregnancies as given in Table 5-16.

Table 5 - 16

Maternal serum pregnancy specific ß-1 glycoprotein (SP_1) medians, in multiples of the median (MOM), and means and standard deviations (SD) of the log_{10} distributions of the controls and Down's syndrome pregnancies.

	n	SP_1 Median (MOM)	Mean	SD
Controls	377	1.00	0.000	0.146
Down's	48	1.17	0.068	0.176



<u>Figure 5 - 15</u>

Maternal serum pregnancy specific ß-1 glycoprotein (SP_1) levels, in multiples of the median (MOM), in 82 pregnancies with chromosome abnormalities compared with the levels in 377 controls.

Table 5 - 17

Maternal serum pregnancy specific ß-1 glycoprotein (SP_1) median values, in multiples of the median (MOM), and significance testing by the Kolmogorov-Smirnov (K-S) test of the distributions for trisomy 18, trisomy 13, unbalanced translocations, balanced translocations and sex chromosome abnormalities, against the control group. (D = absolute difference, p = probability).

	n	SP_1 Median (MOM)	K-S test	
			D	p
Trisomy 18	9	0.87	0.276	>0.2
Trisomy 13	4	1.01	0.258	>0.2
Unbalanced trans.	8	0.52	0.814	<0.01
Balanced trans.	4	0.79	0.413	>0.2
Sex chrom. abn.	9	0.93	0.144	>0.2

translocations showed a significant decrease in SP_1 levels, with all values being below 0.74 MOM, with a median value of 0.52 MOM ($p<0.01$). There was no significant difference between the controls and the nine trisomy 18 pregnancies, the four trisomy 13 pregnancies, the four balanced translocations or the nine sex chromosome abnormalities. The proportions of trisomy 18 pregnancies with SP_1 levels greater than or equal to selected cut-off levels are shown in Table 5-15, with, for example, 4 out of 9 cases (44.4%) having values greater than or equal to 1.0 MOM, compared with 190 out of 377 controls (50.4%) and 34 out of 48 Down's syndrome pregnancies (70.8%).

5.2.1.3 UNCONJUGATED ESTRIOL

5.2.1.3.1 UE3 LEVELS IN UNAFFECTED PREGNANCIES

UE3 levels were analysed as described in Section 4.1.5.1 in 390 control sera taken between 15 and 20 weeks gestation. There were 11 samples at 11 weeks gestation, 184 at 16 weeks, 136 at 17 weeks, 39 at 18 weeks, 10 at 19 weeks and 10 at 20 weeks. Median UE3 values increased with advancing gestation between 15 and 20 weeks. Medians, calculated by linear interpolation were 3.4 nmol/l at 15 weeks, 4.7 nmol/l at 16 weeks, 5.5 nmol/l at 17 weeks, 6.8 nmol/l at 18 weeks, 8.5 nmol/l at 19 weeks and 9.9 nmol/l at 20 weeks.

5.2.1.3.2 UE3 LEVELS IN DOWN'S SYNDROME

The levels of UE3 (in MOM) found in 49 Down's syndrome cases, plotted at the appropriate completed week of gestation, are shown in Figure 5-16. The median value was significantly reduced at 0.79 MOM (95% CL 0.71 - 0.87 MOM) of the unaffected pregnancies ($p<0.02$ by t-test on unpaired variables). The observed numbers and proportion of Down's syndrome and unaffected pregnancies with UE3 levels less than or equal to specific UE3 values are shown in Table 5-18, with, for example, 41 out of 49 of the Down's pregnancies (84%) having UE3 values less than or equal to 1.0 MOM, compared with 202 out of 390 of the unaffected pregnancies (52%).

The distribution of UE3 in the Down's syndrome and unaffected pregnancies was tested for fit to both a Gaussian and a log Gaussian distribution. Means and standard deviations of either a Gaussian or log Gaussian distribution were estimated as described in Section 4.2.3.1 and are shown in Table 5-19. The fit of the individual UE3 values to the distributions predicted by these means and standard deviations assessed by the Kolmogorov-Smirnov Test (Table 5-19). Figure 5-17 shows probability plots comparing the observed values, both on a linear scale (Figure 5-17a) and on a log scale (Figure 5-17b) with the predicted Gaussian or log Gaussian distributions. The observed values, especially those from the unaffected pregnancies, fit a Gaussian distribution better than a log Gaussian distribution. The Gaussian distributions of UE3 in the Down's and control samples are shown in Figure 5-18. The intersection point of the two curves is 0.86 MOM.

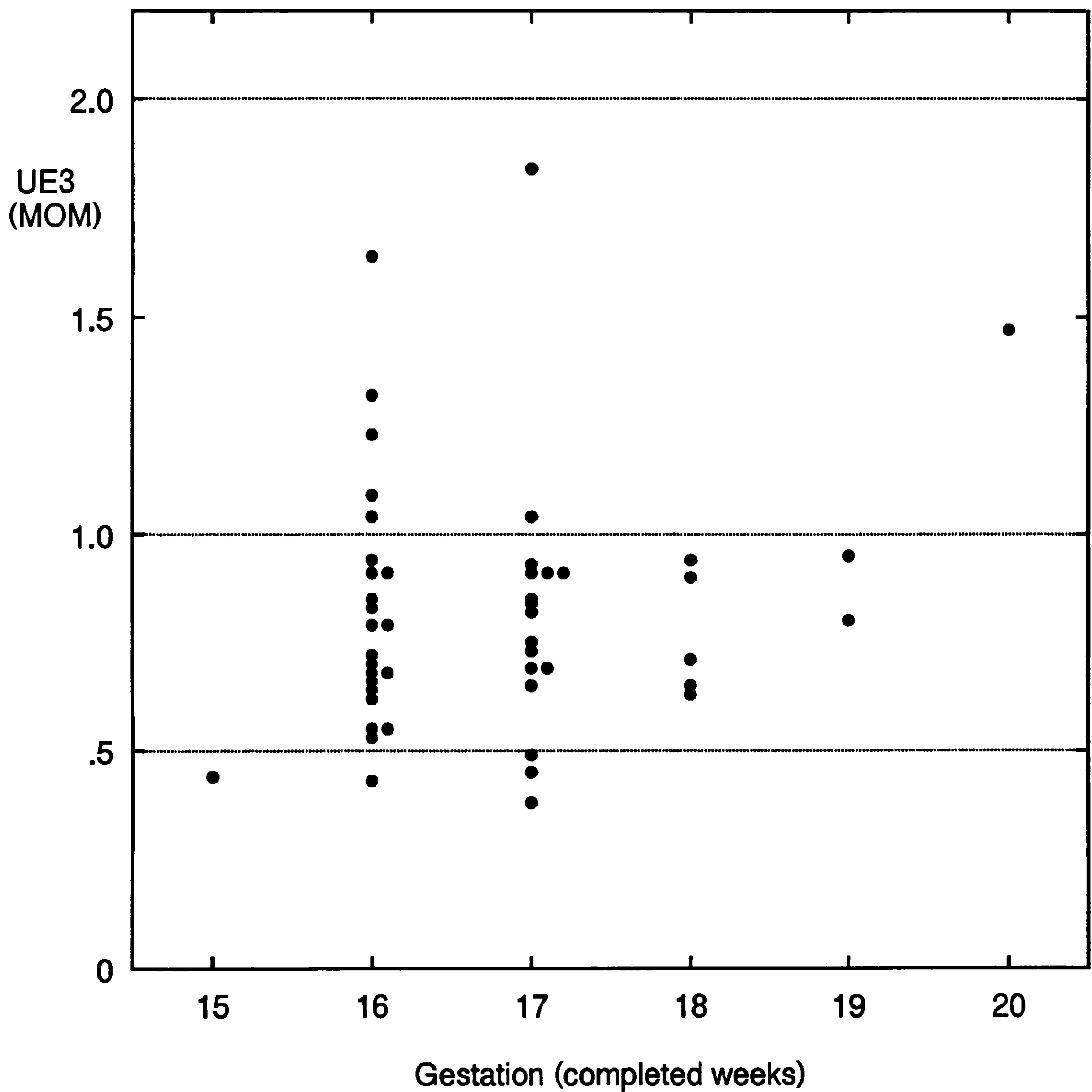


Figure 5 - 16

Maternal serum unconjugated estriol (UE3) levels, in multiples of the median (MOM), in 49 Down's syndrome pregnancies compared with the levels in 390 controls.

Table 5 - 18

Proportion of control and Down's syndrome samples with maternal serum unconjugated estriol (UE3) values, in multiples of the median (MOM), equal to or less than selected cut-off levels.

UE3 (MOM)	Controls (n=390)		Down's (n=49)	
	%	(n)	%	(n)
≤1.9	99	(386)	100	(49)
≤1.6	96	(376)	96	(47)
≤1.3	85	(332)	92	(45)
≤1.0	52	(202)	84	(41)
≤0.7	15	(58)	39	(19)
≤0.4	1	(5)	2	(1)

Table 5 - 19

Parameters of Gaussian or log Gaussian distributions of maternal serum unconjugated estriol values in Down's syndrome and control samples. Kolmorogov-Smirnov (K-S) testing indicates that the Gaussian distribution is the best fit. (D = absolute difference, p = probability)

		Down's (n=49)	Controls (n=390)
Gaussian	Mean:	0.79	1.00
	Standard Deviation:	0.29	0.29
K-S test	D:	0.124	0.036
	p:	0.44	0.69
Log Gaussian	Mean:	-0.102	0.0
	Standard Deviation:	0.156	0.130
K-S test	D:	0.138	0.057
	p:	0.31	0.16

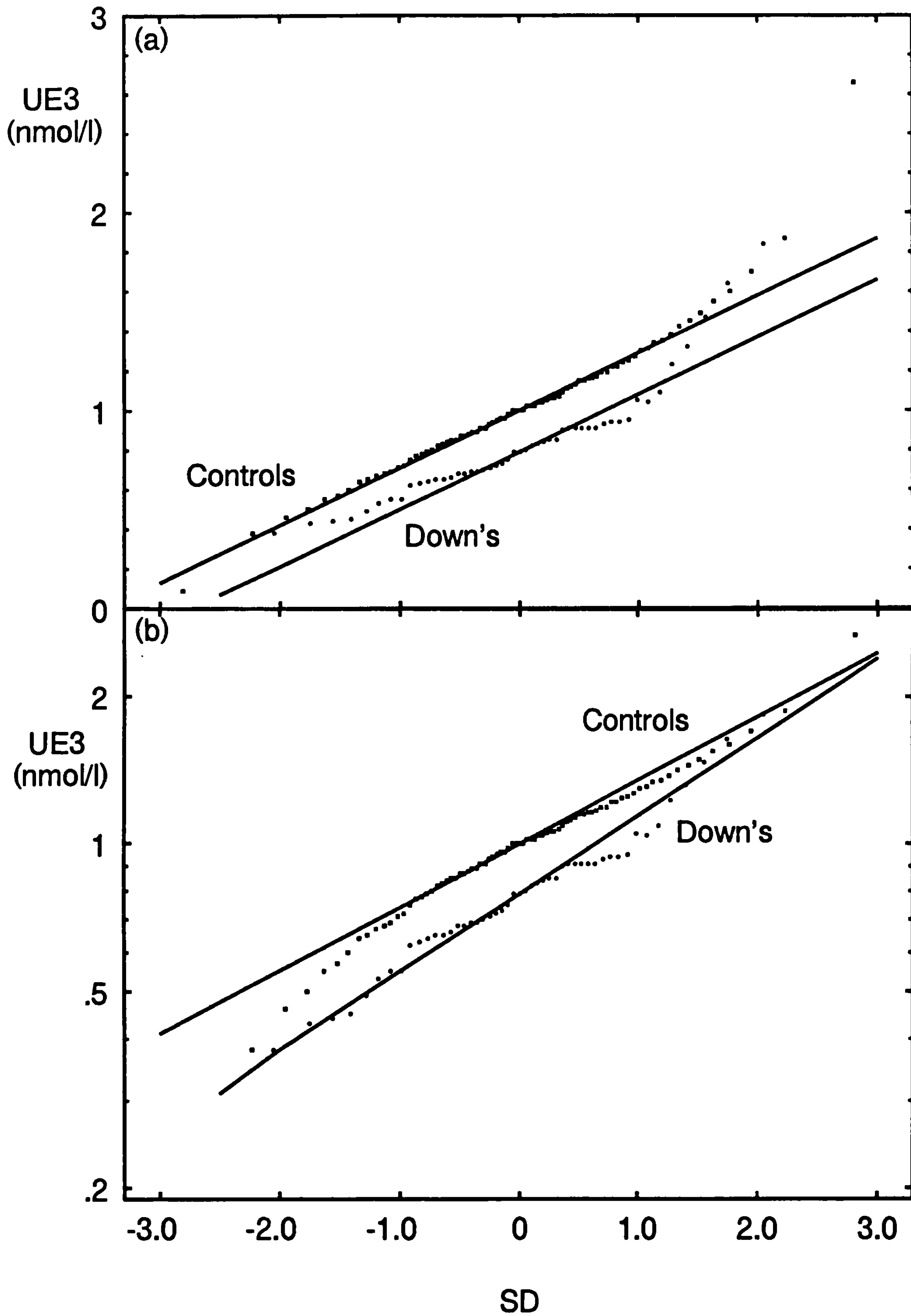


Figure 5 - 17

Probability plot of maternal serum unconjugated estriol (UE3) levels, in multiples of the median (MOM), on (a) a linear scale and (b) a log scale in selected controls and in Down's syndrome pregnancies. The continuous lines are those defined by either Gaussian or log Gaussian distributions with means and standard deviations as defined in Table 5-19.

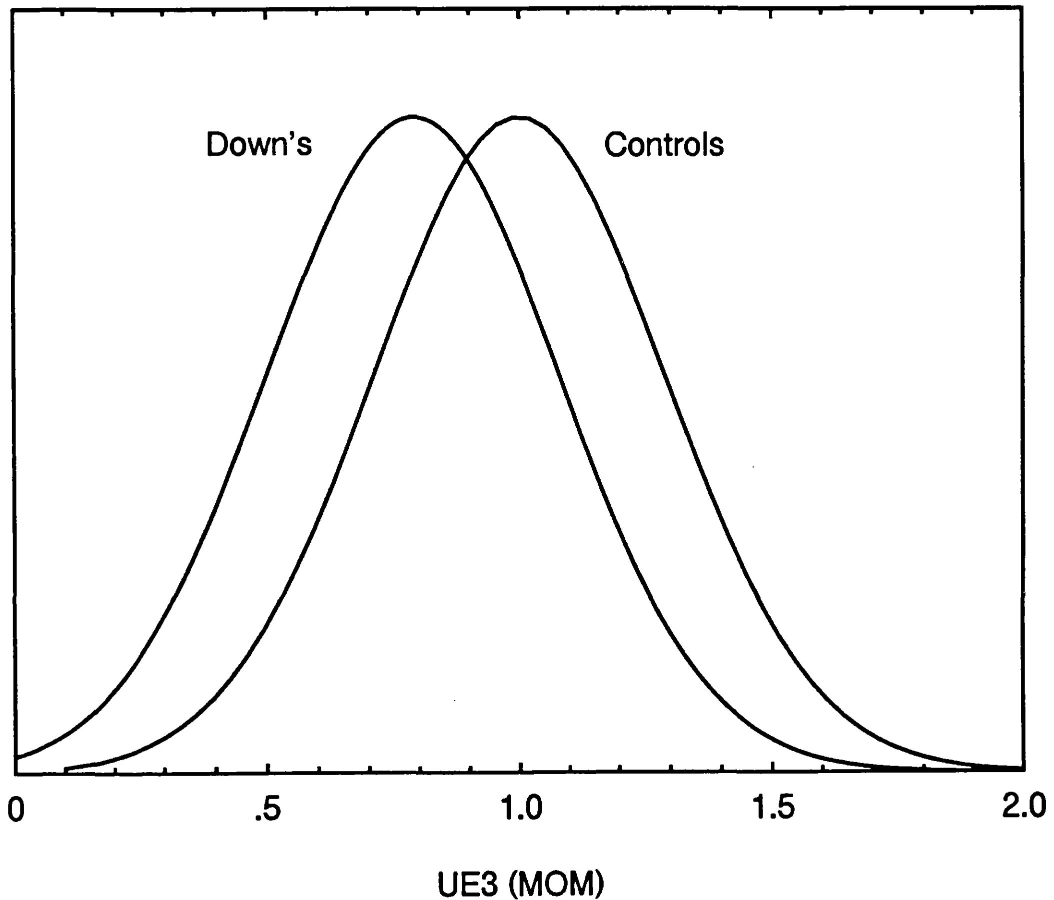


Figure 5 - 18

Gaussian distributions of maternal serum unconjugated estriol (UE3) levels, in multiples of the median (MOM), in controls and Down's syndrome pregnancies. These are plotted from the means and standard deviations of 390 unaffected and 49 Down's syndrome pregnancies as given in Table 5-19.

5.2.1.3.3 UE3 LEVELS IN OTHER CHROMOSOME ABNORMALITIES

The levels of UE3, in MOM, found in maternal serum from the other types of chromosomally abnormal pregnancies are shown on Figure 5-19 and their median values in Table 5-20. Differences between the distributions of the UE3 levels in the control samples and the different types of chromosome abnormality were assessed by the Kolmogorov-Smirnov test (Table 5-20). A significant reduction in UE3 levels was found in four trisomy 18 pregnancies ($p<0.01$), all values being below 0.7 MOM, with a median value of 0.38 MOM. Some lowering of UE3 levels was found in the eight cases of unbalanced translocation ($0.05>p>0.01$) but there was no significant difference between the controls and the other types of chromosome abnormality.

5.2.1.4 FREE ß SUBUNIT OF hCG

5.2.1.4.1 Frß-hCG LEVELS IN UNAFFECTED PREGNANCIES

Frß-hCG was assayed as described in Section 4.1.6.1 in 390 control samples taken between 15 and 20 weeks of gestation. There were 11 samples at 15 weeks gestation, 184 at 16 weeks, 136 at 17 weeks, 39 at 18 weeks, 10 at 19 weeks and 10 at 20 weeks. Maternal serum Frß-hCG levels declined slightly between 15 and 20 weeks gestation. Median values, calculated by linear interpolation, were 6.5 IU/l at 15 weeks, 6.1 IU/l at 16 and 17 weeks, 5.4 IU/l at 18 weeks, 4.8 IU/l at 19 weeks and 4.3 IU/l at 20 weeks.

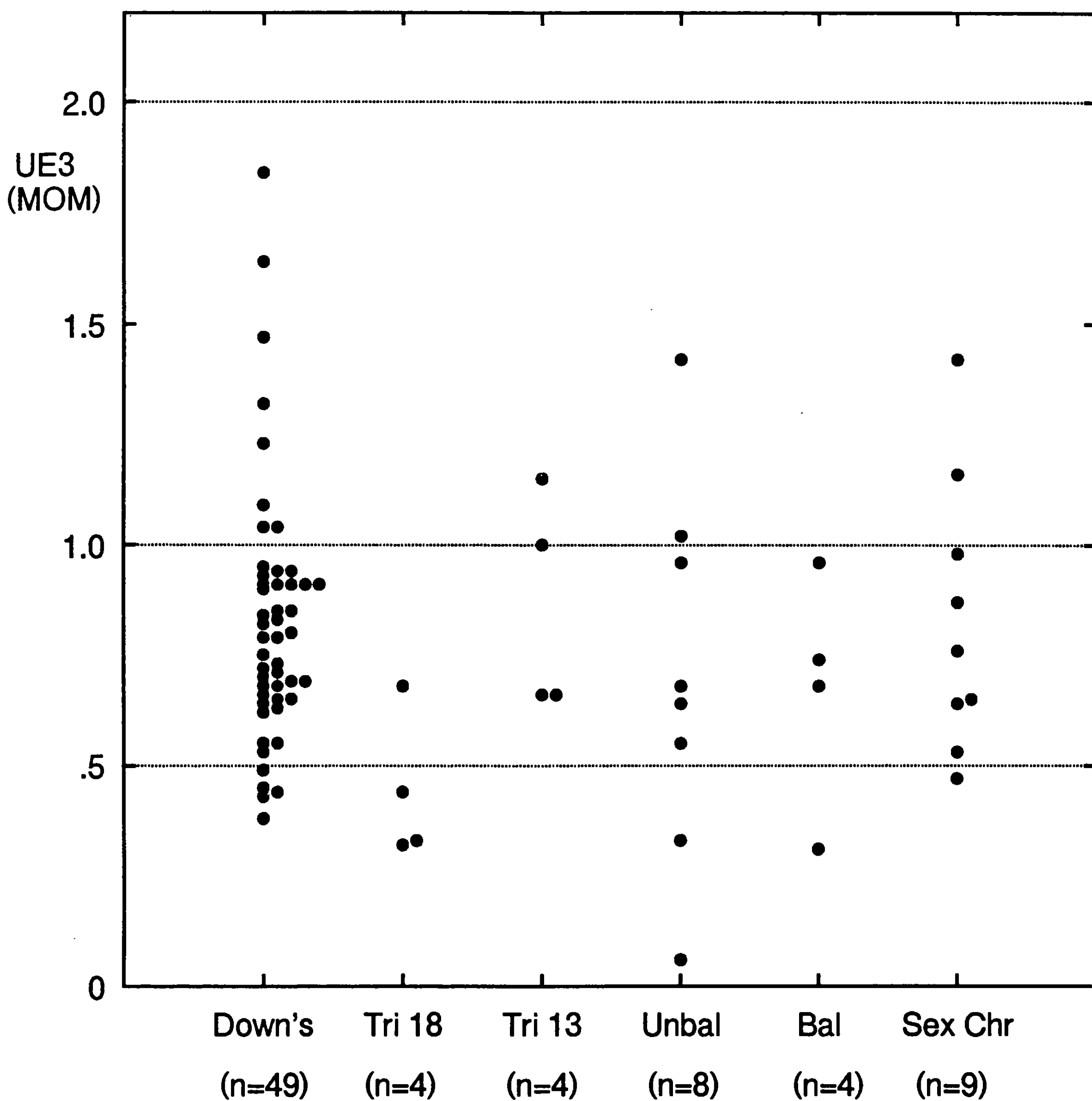


Figure 5 - 19

Maternal serum unconjugated estriol (UE3) levels, in multiples of the median (MOM), in 78 chromosomally abnormal pregnancies compared with the levels in 390 unaffected controls.

Table 5 - 20

Maternal serum unconjugated estriol (UE3) median values, in multiples of the median (MOM), and significance testing by the Kolmorogov-Smirnov (K-S) test of the distributions of UE3 levels for trisomy 18, trisomy 13, unbalanced translocations, balanced translocations and sex chromosome abnormalities, against the control group.

	n	UE3 Median (MOM)	K-S test	
			D	p
Trisomy 18	4	0.38	0.864	0.005
Trisomy 13	4	0.83	0.380	>0.1
Unbalanced trans.	8	0.66	0.489	0.043
Balanced trans.	4	0.71	0.569	>0.1
Sex chrom. abn.	9	0.77	0.347	>0.1

5.2.1.4.2 Frβ-hCG LEVELS IN DOWN'S SYNDROME

The levels of Frβ-hCG, in MOM, in maternal serum from 81 cases of Down's syndrome, plotted at the appropriate completed week of gestation, are shown in Figure 5-20. The numbers and percentages of Down's syndrome cases and controls with Frβ-hCG levels greater than or equal to selected cut-off levels are shown in Table 5-21, with, for example 48 out of 81 of the Down's syndrome pregnancies (60.5%) having Frβ-hCG levels greater than 2.0 MOM, compared with 63 out of 390 of the controls (16.2%). The median value of the Down's syndrome cases were found to be significantly elevated at 2.30 MOM (95% CL 1.92 to 2.75 MOM) of the controls ($p<0.001$ by t-test on unpaired variables). A probability plot (Figure 5-21) shows that the distribution of the Frβ-hCG values in the controls above 0.3 MOM and in the Down's syndrome samples above 0.7 MOM fit log Gaussian distributions (Figure 5-22). The intersection point of the two curves is 1.60 MOM. Means and standard deviations of the controls and Down's syndrome Frβ-hCG distributions were estimated as described in Section 4.2.3.1 and are shown in Table 5-22.

5.2.1.4.3 Frβ-hCG LEVELS IN OTHER CHROMOSOME ABNORMALITIES

The levels of Frβ-hCG, in MOM, in sera from the other chromosomally abnormal pregnancies are shown in Figure 5-23. Medians for the 11 cases of trisomy 18, four cases of trisomy 13, 10 unbalanced translocations, four balanced translocations and a heterogeneous group of nine sex chromosome abnormalities are shown

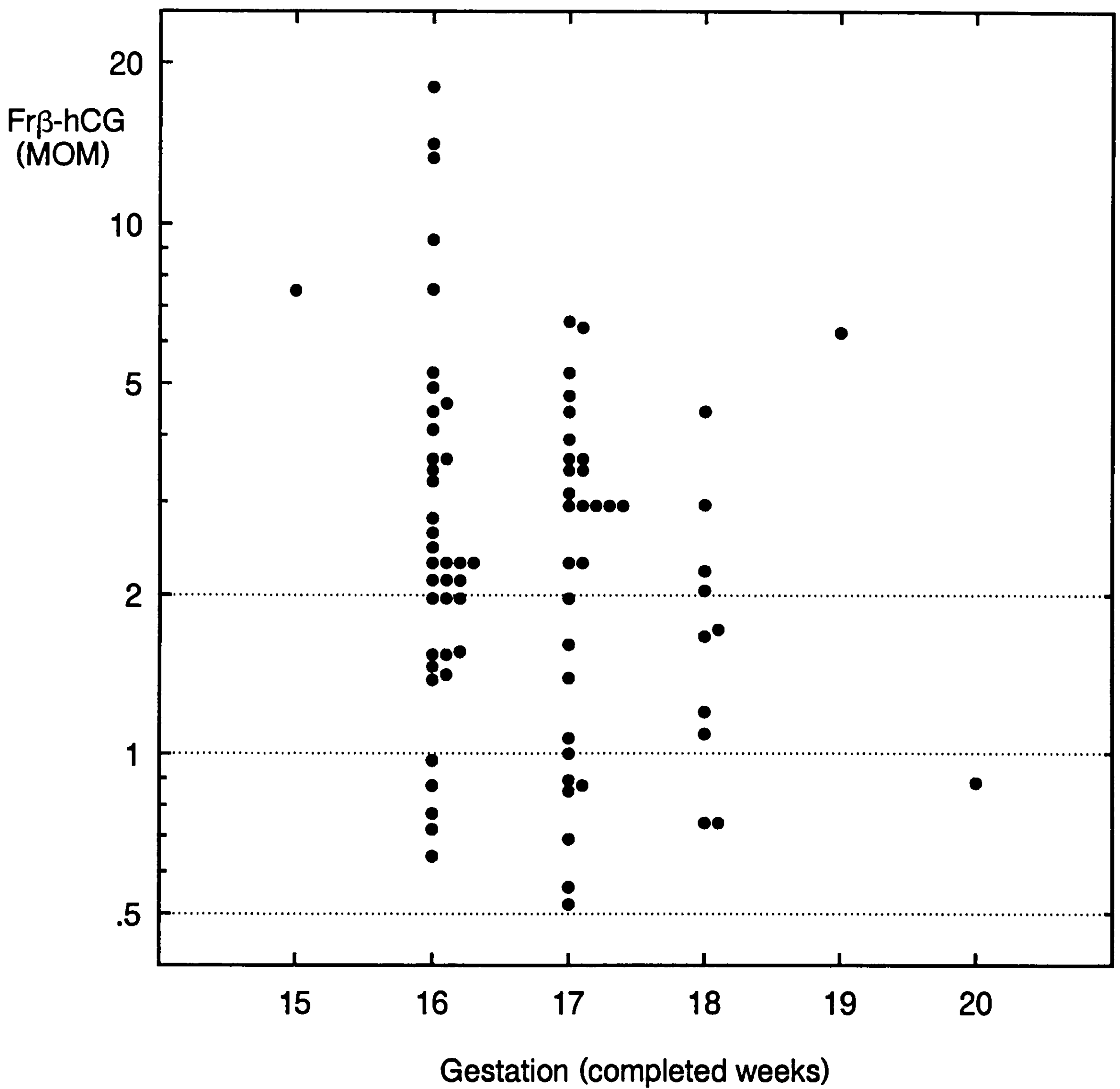


Figure 5 - 20

Maternal serum free β subunit hCG (Frβ-hCG) levels, in multiples of the median (MOM) in 81 Down's syndrome pregnancies compared with the levels in 390 unaffected controls.

Table 5 - 21

Proportion of control, Down's syndrome and trisomy 18 samples with maternal serum free ß subunit hCG (Frß-hCG) levels, in multiples of the median (MOM), greater than or equal to selected cut-off values.

Frß-hCG (MOM)	Controls (n=390)		Down's (n=81)		Trisomy 18 (n=11)	
	%	(n)	%	(n)	%	(n)
≥3.0	5.6	(22)	34.6	(28)	0	(0)
≥2.5	9.5	(37)	44.4	(36)	0	(0)
≥2.0	16.2	(63)	60.5	(49)	0	(0)
≥1.5	26.7	(104)	72.8	(59)	0	(0)
≥1.0	49.7	(194)	82.7	(67)	0	(0)
≥0.5	83.8	(327)	100	(81)	18.2	(2)

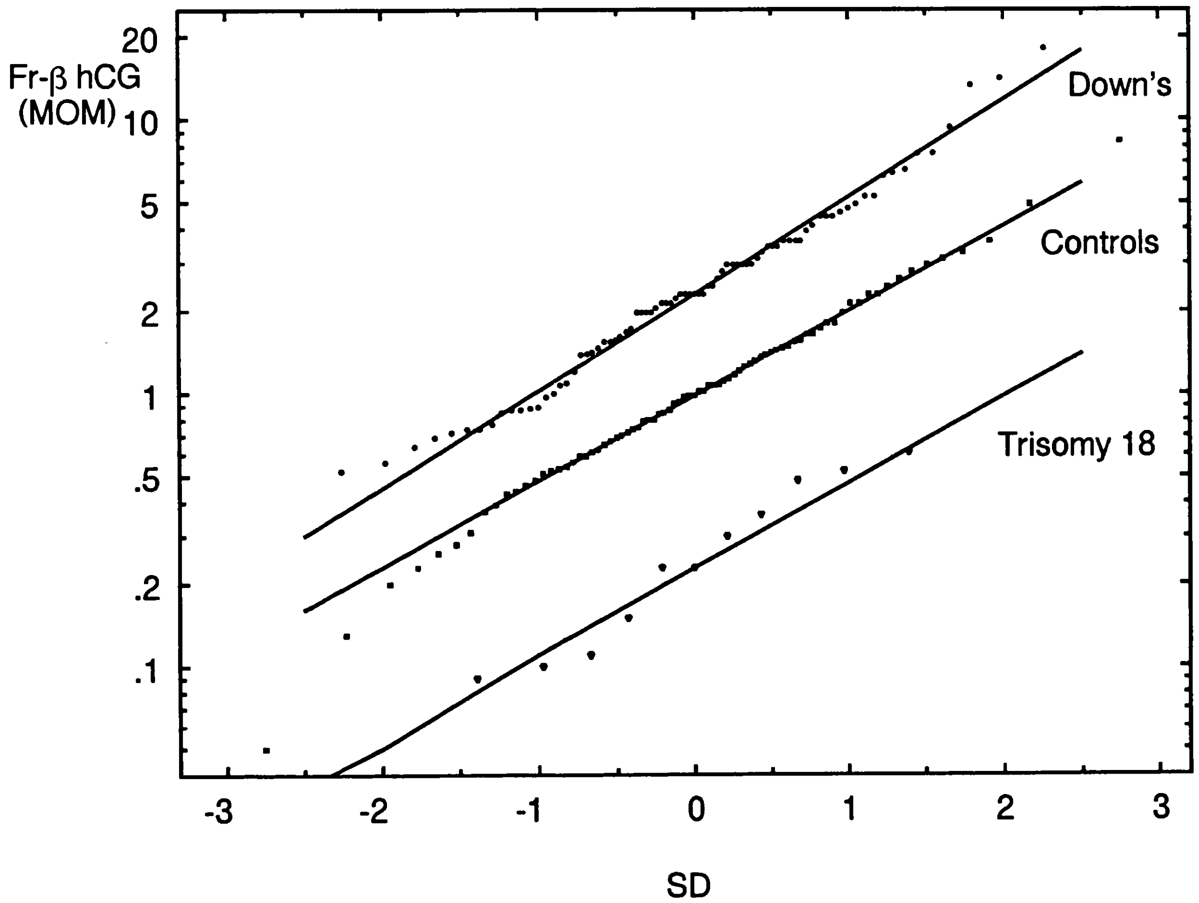


Figure 5 - 21

Probability plot of maternal serum free β subunit hCG (Frβ-hCG), in multiples of the median (MOM), on a log scale, in selected controls, Down's syndrome pregnancies and trisomy 18 pregnancies. The continuous lines are those defined by log Gaussian distributions with means and standard deviations as given in Table 5-22.

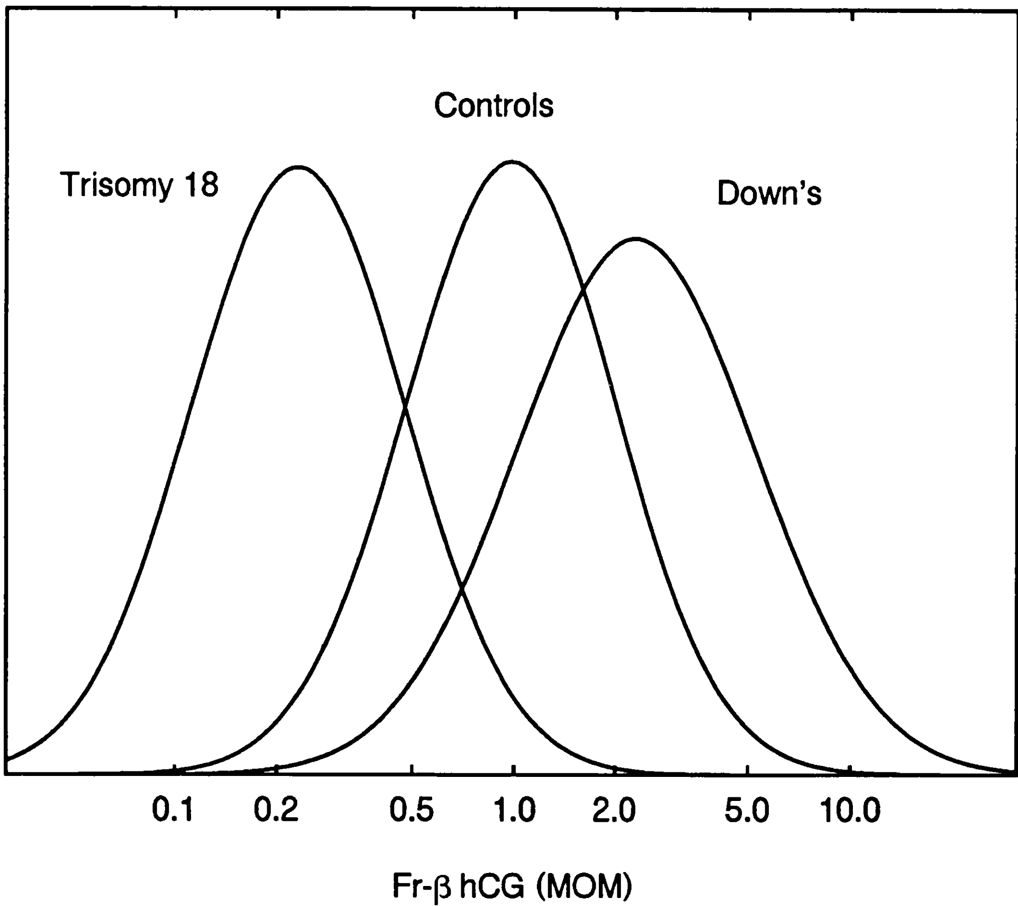


Figure 5 - 22

Log Gaussian distributions of maternal serum free β subunit hCG (Frβ-hCG) levels, in multiples of the median (MOM), in controls, Down's syndrome pregnancies and trisomy 18 pregnancies. These are plotted from the means and standard deviations of 390 unaffected, 81 Down's syndrome and 11 Trisomy 18 pregnancies as given in Table 5-22.

Table 5 - 22

Maternal serum free ß subunit hCG (Frß-hCG) medians, in multiples of the median (MOM), and means and standard deviations (SD) of the $\log_{10}$ distribution for controls, Down's syndrome pregnancies and trisomy 18 pregnancies.

	n	Median (MOM)	Mean	SD
Controls	390	0.98	-0.009	0.311
Down's	81	2.30	0.362	0.355
Trisomy 18	11	0.23	-0.638	0.314

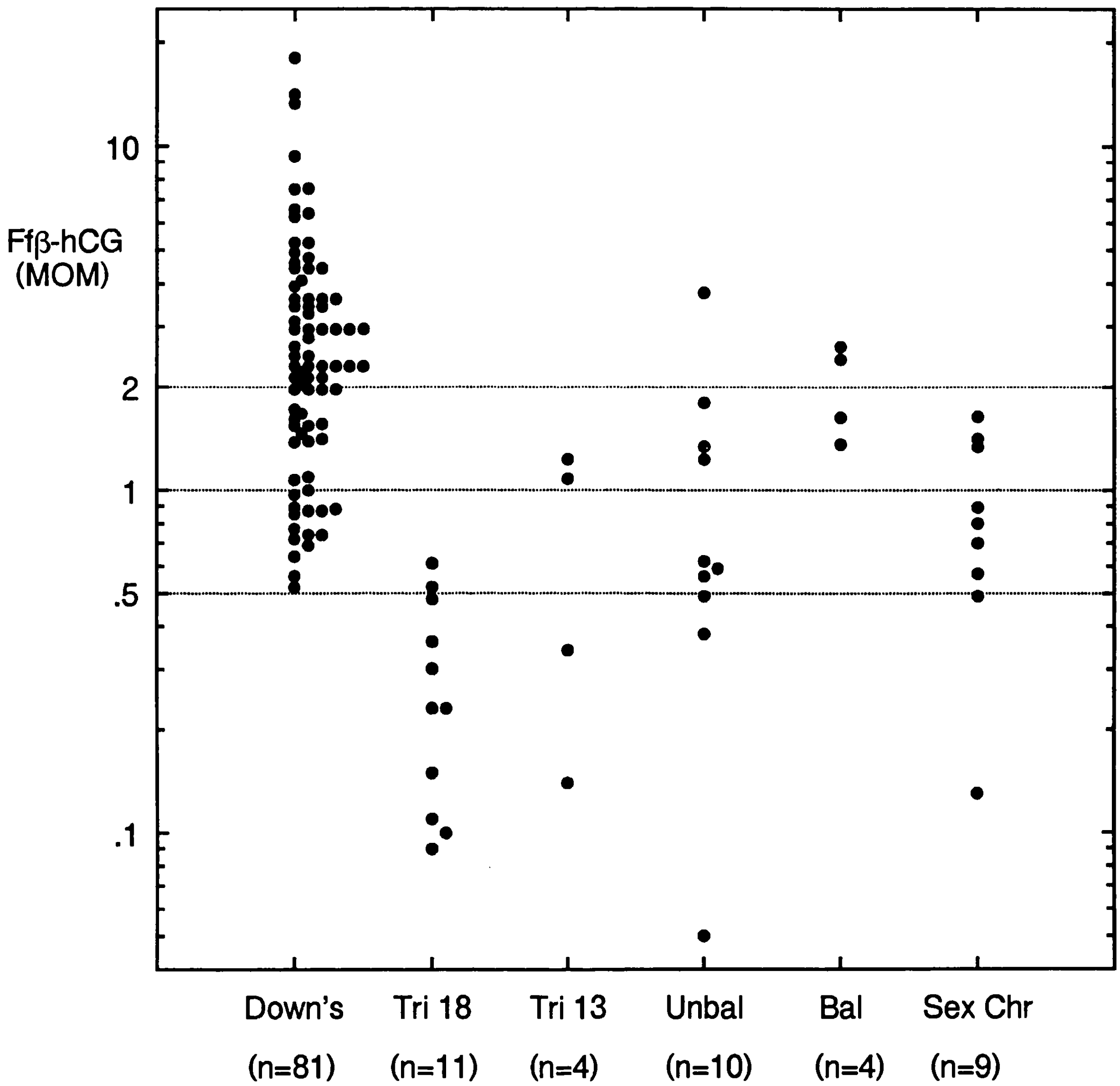


Figure 5 - 23

Maternal free ß subunit hCG (Frß-hCG) levels, in multiples of the median (MOM), in 119 chromosomally abnormal pregnancies compared with the levels in 390 controls.

in Table 5-23. Differences between the distributions of Frβ-hCG levels in the controls samples and these various types of chromosome abnormality were assessed using the Kolmogorov-Smirnov test (Table 5-23). There was no significant difference between the controls and the trisomy 13 pregnancies, the unbalanced translocations, the balanced translocation or the sex chromosome abnormalities. The 11 trisomy 18 pregnancies show a significant decrease in Frβ-hCG levels, with all values being below 0.65 MOM, with a median value of 0.23 MOM ($p<0.001$).

Although the numbers are small, the trisomy 18 Frβ-hCG values appear to fit a log Gaussian distribution (See probability plot, Figure 5-21). The log Gaussian distribution of Frβ-hCG in the trisomy 18 samples, with mean and standard deviation as given in Table 5-22, appears to have a greater shift away from the levels in the controls than the Down's syndrome samples (Figure 5-22). The proportions of trisomy 18 samples with levels greater than or equal to selected cut-off levels are shown in Table 5-21, with only 2 cases out of 11 (18.2%) having a value greater than or equal to 0.5 MOM, compared with 327 out of 390 controls (83.8%) and all 81 Down's syndrome samples (100%).

5.2.2 CORRELATION BETWEEN ANALYTES

The levels of correlation between analytes in controls and Down's syndrome samples were assessed by calculating the product-moment correlation coefficient (r), using the appropriate multiples of the median (MOM) or $\log_{10}$MOM, depending on whether the data fitted

Table 5 - 23

Maternal serum free ß subunit hCg (Frß-hCG) median values, in multiples of the medain (MOM), and significance testing by the Kolmogorov-Smirnov (K-S) test of the distributions of Frß-hCG levels in trisomy 18, trisomy 13, unbalanced translocations, balanced translocations and sex chromosome abnormalities against the control group. (D = absolute difference, p = probability).

	n	Frß-hCG Median (MOM)	K-S test D	K-S test p
Trisomy 18	11	0.23	0.7483	<0.001
Trisomy 13	4	0.71	0.4279	>0.2
Unbalanced trans.	10	0.61	0.3363	>0.2
Balanced Trans.	4	2.02	0.6769	0.051
Sex chrom abn.	9	0.80	0.2360	>0.2

a Gaussian or log Gaussian distribution. The values of r for controls and Down's syndrome samples for combination of analytes and also maternal age are shown in Table 5-24.

Highly significant correlations ($p<0.01$) were found in both the Down's syndrome and control samples between UE3 and AFP, between SP_1 and hCG, between SP_1 and Frß-hCG and between hCG and Frß-hCG, and, in the control samples only, between SP_1 and UE3.

Lower levels of correlation ($0.05>p>0.01$) were found in the control samples only between hCG and AFP, between UE3 and hCG and between UE3 and Frß-hCG.

No significant correlation was found between AFP and Frß-hCG and between AFP and SP_1 nor between any of the analytes and maternal age.

5.2.3 SUMMARY OF ANALYTE RESULTS

In Down's syndrome all five markers studied, AFP, hCG, SP_1, UE3 and Frß-hCG show a shift in mean value away from that of the control distribution, AFP and UE3 having reduced levels and hCG, SP_1 and Frß-hCG having increased levels. The comparative shifts of the Down's syndrome mean away from the control group mean, measured in standard deviations, are shown in Table 5-25. The relative shifts are hCG > Frß-hCG > AFP > UE3 > SP_1.

Trisomy 18 pregnancies show lowered median levels for all five

Table 5 - 24

Correlation coefficients (r) for unaffected and Down's syndrome pregnancies between alphafetoprotein (AFP), unconjugated estriol (UE3), free ß subunit hCG (Frß-hCG), pregnancy specific ß-1 glycoprotein (SP_1) and maternal age.

CORRELATION COEFFICIENT (r) - UNAFFECTED
- DOWN'S

	UE3	$logSP_1$	logHCG	logFrß-HCG	Age
logAFP	0.25** 0.44**	0.10 0.23	0.11* 0.12	0.07 0.08	0.03 0.14
UE3		0.19** 0.22	-0.13* 0.11	-0.14* 0.06	0.01 -0.24
$logSP_1$			0.29** 0.35**	0.15** 0.29*	0.07 0.06
logHCG				0.82** 0.74**	-0.03 -0.10
logFrß-hCG					-0.01 0.02

** Highly significant correlation ($p<0.01$)

* Significant correlation ($0.01<p<0.05$)

Table 5 - 25

Summary, for different analytes, of the shift in mean of the Down's syndrome distribution away from that of the control distribution. The shift for each analyte was derived by dividing the difference between the mean of the control and Down's syndrome distribution by the standard deviation (SD) of the control distribution.

Analyte	Shift in mean of Down's distribution (SD)
SP_1	0.47
UE3	0.72
AFP	0.83
Frβ-hCG	1.19
hCG	1.34

analytes studied, although for SP_1 the reduction is not statistically significant. The median values for trisomy 18 pregnancies are summarised in Table 5-26. The relative lowering is hCG < Frß-hCG < UE3 < AFP < SP_1.

Of the other types of chromosome abnormality only the unbalanced translocations show some lowering of hCG and UE3 levels and significant lowering of SP_1 levels. These unbalanced translocations are however a heterogeneous group.

5.2.4 USE OF PREGNANCY MARKERS IN SCREENING FOR CHROMOSOME ABNORMALITIES

5.2.4.1 USE OF INTACT hCG IN SCREENING FOR CHROMOSOME ABNORMALITIES

There is a small level of correlation (Table 5-24) between AFP and intact hCG ($0.01<p<0.05$) and the correlation between either analyte and maternal age is not significantly different from zero ($p>0.05$). Therefore, the information which can be derived from each variable with respect to the identification of Down's syndrome pregnancies is virtually independent and may be used in the calculation of a combined risk factor. A risk factor for an individual pregnancy can be calculated using maternal age and either hCG alone or hCG and AFP combined. Maternal serum hCG levels are more discriminating than either age or AFP levels alone or in combination for the detection of Down's syndrome pregnancies (Table 5-27). Since raised hCG levels are found only in Down's

Table 5 - 26

Summary of median analyte levels in trisomy 18 pregnancies, in multiples of the median (MOM).

Analyte	Trisomy 18 median (MOM)
hCG	0.21
Frß-hCG	0.23
UE3	0.38
AFP	0.68
SP_1	0.87

Table 5 - 27

Comparison of false positive rates for human chorionic gonadotrophin (hCG), alphafetoprotein (AFP) and maternal age at different detection rates.

	Detection rate (%) for Down's syndrome	False positive rate (%)	Threshold
hCG	60	14	≥1.8 MOM
	30	2.2	≥2.8 MOM
AFP	60	28	≤0.8 MOM
	30	9.0	≤0.6 MOM
Age	60	37	≥28 years
	30	6.7	≥35 years

syndrome pregnancies and not in other autosomal trisomies, any screening policy which makes use of these elevated hCG levels will detect only the trisomy 21 cases.

5.2.4.2 USING hCG AND AGE TO SCREEN FOR DOWN'S SYNDROME

Likelihood ratios were calculated from the overlapping Gaussian distributions of hCG levels in Down's syndrome and unaffected pregnancies as described in Section 4.2.8, using the means and standard deviations given in Table 5-13. A combined risk of Down's syndrome for an individual pregnancy, based on hCG and age was calculated by multiplying the left hand side of the odds ratio of having a Down's syndrome fetus at mid-trimester (Table 5-3) by the likelihood ratio. Table 5-28 gives combined risks of Down's syndrome for individual pregnancies between 15-20 weeks gestation. Detection and false positive rates were estimated in the west of Scotland pregnant population as described in Section 4.2.9. Table 5-29 shows the effect of using different mid-trimester risk thresholds on the detection and false positive rates, with for example, a predicted detection rate for Down's syndrome of 51% at a corresponding 5% false positive rate using a mid-trimester cut-off risk of 1:310. The variation in detection and false positive rates between different maternal age groups is shown in Table 5-30 with, for example, in the age group 20-24 years, a predicted detection rate for Down's syndrome of 29% and a corresponding false positive rate of 1.6%, compared with 68% detection and a 22% false positive rate in the age group 35-37 years.

Table 5 - 28

Risk estimates of finding a Down's syndrome fetus at mid-trimester, based on maternal age and maternal serum chorionic gonadotrophin (hCG) at 15-20 weeks gestation (MOM = multiples of the median).

Mat. Age (yrs)	hCG(MOM) 3.2	3.1	3.0	2.9	2.8	2.7	2.6	2.5	2.4	2.3	2.2	2.1	2.0
16	1: 240	1: 270	1: 290	1: 320	1: 350	1: 380	1: 420	1: 460	1: 510	1: 570	1: 630	1: 700	1: 780
18	1: 240	1: 260	1: 290	1: 310	1: 340	1: 380	1: 420	1: 460	1: 510	1: 560	1: 620	1: 700	1: 770
20	1: 240	1: 260	1: 280	1: 310	1: 340	1: 370	1: 410	1: 450	1: 500	1: 550	1: 610	1: 680	1: 760
22	1: 230	1: 250	1: 270	1: 300	1: 330	1: 360	1: 400	1: 440	1: 480	1: 530	1: 590	1: 660	1: 740
24	1: 220	1: 240	1: 260	1: 280	1: 310	1: 340	1: 380	1: 410	1: 460	1: 510	1: 560	1: 630	1: 700
26	1: 200	1: 220	1: 240	1: 260	1: 280	1: 310	1: 340	1: 380	1: 420	1: 460	1: 510	1: 570	1: 640
27	1: 190	1: 210	1: 230	1: 250	1: 280	1: 300	1: 330	1: 370	1: 400	1: 450	1: 500	1: 560	1: 620
28	1: 170	1: 190	1: 210	1: 230	1: 250	1: 270	1: 300	1: 330	1: 260	1: 400	1: 450	1: 500	1: 560
29	1: 160	1: 170	1: 190	1: 210	1: 220	1: 250	1: 270	1: 300	1: 330	1: 370	1: 410	1: 450	1: 570
30	1: 140	1: 150	1: 170	1: 180	1: 200	1: 220	1: 240	1: 270	1: 300	1: 330	1: 360	1: 410	1: 450
31	1: 120	1: 130	1: 150	1: 160	1: 180	1: 190	1: 210	1: 240	1: 260	1: 290	1: 320	1: 360	1: 400
32	1: 110	1: 120	1: 130	1: 140	1: 150	1: 170	1: 180	1: 200	1: 200	1: 250	1: 270	1: 310	1: 340
33	1: 89	1: 100	1: 110	1: 120	1: 130	1: 140	1: 150	1: 170	1: 190	1: 210	1: 230	1: 260	1: 290
34	1: 73	1: 80	1: 87	1: 95	1: 100	1: 120	1: 130	1: 140	1: 150	1: 170	1: 190	1: 210	1: 240
35	1: 59	1: 65	1: 71	1: 77	1: 85	1: 93	1: 100	1: 110	1: 120	1: 140	1: 150	1: 170	1: 190
36	1: 48	1: 52	1: 57	1: 62	1: 68	1: 75	1: 82	1: 91	1: 100	1: 110	1: 120	1: 140	1: 150
37	1: 38	1: 41	1: 45	1: 49	1: 54	1: 59	1: 65	1: 72	1: 79	1: 87	1: 97	1: 110	1: 120
38	1: 29	1: 32	1: 35	1: 38	1: 42	1: 46	1: 51	1: 56	1: 61	1: 68	1: 76	1: 84	1: 94
39	1: 23	1: 25	1: 27	1: 29	1: 32	1: 36	1: 39	1: 43	1: 48	1: 53	1: 59	1: 65	1: 73
40	1: 17	1: 19	1: 21	1: 23	1: 25	1: 27	1: 30	1: 33	1: 37	1: 41	1: 45	1: 50	1: 56
41	1: 13	1: 14	1: 16	1: 17	1: 19	1: 21	1: 23	1: 25	1: 28	1: 31	1: 34	1: 38	1: 42
42	1: 10	1: 11	1: 12	1: 13	1: 14	1: 16	1: 17	1: 19	1: 21	1: 23	1: 26	1: 29	1: 32
43	1: 7	1: 8	1: 9	1: 10	1: 11	1: 12	1: 13	1: 14	1: 16	1: 18	1: 20	1: 22	1: 24
44	1: 6	1: 6	1: 7	1: 8	1: 8	1: 9	1: 10	1: 11	1: 12	1: 14	1: 15	1: 17	1: 19
45	1: 4	1: 5	1: 5	1: 6	1: 6	1: 7	1: 7	1: 8	1: 9	1: 10	1: 11	1: 12	1: 14

Mat. Age (yrs)	hCG(MOM) 1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.0	0.8	0.6	0.4
16	1: 880	1: 980	1:1100	1:1300	1:1400	1:1600	1:1900	1:2100	1:2500	1:2800	1:3800	1:5200	1:7000
18	1: 870	1: 970	1:1100	1:1200	1:1400	1:1600	1:1800	1:2100	1:2400	1:2800	1:3800	1:5200	1:6900
20	1: 850	1: 950	1:1100	1:1200	1:1400	1:1600	1:1800	1:2100	1:2400	1:2700	1:3700	1:5100	1:6800
22	1: 830	1: 930	1:1000	1:1200	1:1300	1:1500	1:1700	1:2000	1:2300	1:2600	1:3600	1:4900	1:6600
24	1: 790	1: 880	1: 990	1:1100	1:1300	1:1500	1:1700	1:1900	1:2200	1:2500	1:3400	1:4700	1:6200
26	1: 720	1: 800	1: 910	1:1000	1:1200	1:1300	1:1500	1:1700	1:2000	1:2300	1:3100	1:4300	1:5700
27	1: 700	1: 780	1: 880	1:1000	1:1100	1:1300	1:1500	1:1700	1:2000	1:2200	1:3000	1:4200	1:5500
28	1: 630	1: 700	1: 790	1: 900	1:1000	1:1200	1:1300	1:1500	1:1800	1:2000	1:2700	1:3700	1:5000
29	1: 570	1: 640	1: 720	1: 810	1: 930	1:1100	1:1200	1:1400	1:1600	1:1800	1:2500	1:3400	1:4500
30	1: 510	1: 570	1: 640	1: 730	1: 830	1: 940	1:1100	1:1200	1:1400	1:1600	1:2200	1:3000	1:4000
31	1: 450	1: 500	1: 560	1: 640	1: 720	1: 830	1: 940	1:1100	1:1200	1:1400	1:1900	1:2700	1:3500
32	1: 380	1: 430	1: 480	1: 550	1: 620	1: 710	1: 800	1: 930	1:1100	1:1200	1:1700	1:2300	1:3000
33	1: 320	1: 360	1: 410	1: 460	1: 510	1: 600	1: 680	1: 780	1: 900	1:1000	1:1400	1:1900	1:2600
34	1: 270	1: 300	1: 340	1: 380	1: 430	1: 490	1: 560	1: 640	1: 740	1: 840	1:1100	1:1600	1:2100
35	1: 210	1: 240	1: 270	1: 310	1: 350	1: 400	1: 450	1: 520	1: 600	1: 680	1: 930	1:1300	1:1700
36	1: 170	1: 190	1: 220	1: 250	1: 280	1: 320	1: 360	1: 420	1: 480	1: 550	1: 750	1:1000	1:1400
37	1: 140	1: 150	1: 170	1: 190	1: 220	1: 250	1: 290	1: 330	1: 380	1: 430	1: 590	1: 810	1:1100
38	1: 110	1: 120	1: 130	1: 150	1: 170	1: 200	1: 220	1: 260	1: 300	1: 340	1: 460	1: 540	1: 840
39	1: 82	1: 91	1: 100	1: 120	1: 130	1: 150	1: 170	1: 200	1: 230	1: 260	1: 350	1: 490	1: 620
40	1: 63	1: 70	1: 80	1: 90	1: 100	1: 120	1: 130	1: 150	1: 180	1: 200	1: 270	1: 380	1: 500
41	1: 48	1: 53	1: 60	1: 68	1: 77	1: 88	1: 100	1: 120	1: 130	1: 150	1: 210	1: 280	1: 380
42	1: 36	1: 41	1: 46	1: 52	1: 59	1: 68	1: 76	1: 88	1: 100	1: 120	1: 160	1: 220	1: 290
43	1: 27	1: 30	1: 35	1: 39	1: 44	1: 51	1: 57	1: 66	1: 76	1: 87	1: 120	1: 160	1: 220
44	1: 21	1: 23	1: 27	1: 30	1: 34	1: 39	1: 44	1: 51	1: 59	1: 67	1: 91	1: 130	1: 170
45	1: 15	1: 17	1: 19	1: 22	1: 25	1: 29	1: 32	1: 37	1: 43	1: 49	1: 67	1: 92	1: 120

Table 5 - 29

Comparison of detection rates for Down's syndrome and corresponding false positive rates at varying mid-trimester risk thresholds in the west of Scotland pregnant population using hCG/age screening.

Cut-off risk	Detection rate for Down's syndrome (%)	False positive rate (%)
1:250	45	3.4
1:280	47	4.2
1:300	50	4.7
1:310	51	5.0
1:350	54	5.9

Table 5 -30

Detection rates for Down's syndrome and corresponding false positive rates in different maternal age groups using hCG/age and a mid-trimester cut-off risk of 1:310.

Age group	Detection rate (%)	False positive rate (%)
<20	29	1.2
20-24	29	1.6
25-29	35	2.6
30-34	51	6.2
35-37	68	22
38-40	85	54
>40	99	94
Overall	51	5.0

5.2.4.3 USING hCG, AFP AND AGE TO SCREEN FOR DOWN'S SYNDROME

Three possible methods of calculating a combined risk factor, taking into account maternal age, and serum AFP and hCG levels are described below, and the effects on detection rates and corresponding false positive rates compared.

5.2.4.3.1 COMBINING SEPARATE RISKS DERIVED FROM AFP AND hCG LEVELS

The risk (likelihood ratio) that a specific hCG or AFP result is associated with a Down's syndrome pregnancy can be calculated from the overlapping log Gaussian distributions as described in Section 4.2.8. Likelihood ratios for hCG were calculated using the means and standard deviations for Down's syndrome and control pregnancies given in Table 5-13 and for AFP those in Table 5-2. A combined risk factor for hCG and AFP can be derived simply by multiplying together the individual likelihood ratios, but this ignores the small correlation between the two variables. Table 5-31 shows a matrix of likelihood ratios calculated by this method for a range of hCG and AFP levels, and an individual woman's risk can be calculated by incorporating the appropriate population Down's syndrome risk from Table 5-3. The overall risk of having a Down's syndrome fetus at screening between 15-20 weeks can be calculated by multiplying the left hand side of the maternal age specific risk by the combined likelihood ratio.

Table 5 - 31

Combined likelihood ratios for Down's syndrome for AFP and hCG, ignoring the correlation between variables.

AFP (MOM)	hCG(MOM) 0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8
0.35	1.06	1.13	1.31	1.50	1.75	2.07	2.38	2.82	3.19	3.69	4.26	4.82	5.51	6.26	7.07	8.01
0.40	0.82	0.87	1.02	1.16	1.36	1.60	1.84	2.18	2.45	2.86	3.29	3.73	4.26	4.84	5.47	6.20
0.45	0.65	0.69	0.81	0.92	1.08	1.27	1.46	1.73	1.96	2.27	2.61	2.96	3.38	3.84	4.34	4.92
0.50	0.53	0.56	0.65	0.74	0.87	1.02	1.18	1.40	1.58	1.83	2.11	2.39	2.73	3.10	3.50	3.97
0.55	0.43	0.46	0.54	0.61	0.71	0.84	0.97	1.15	1.30	1.50	1.73	1.96	2.24	2.55	2.88	3.26
0.60	0.36	0.38	0.45	0.51	0.60	0.70	0.81	0.96	1.09	1.26	1.45	1.64	1.87	2.13	2.41	2.73
0.65	0.31	0.32	0.38	0.43	0.50	0.59	0.68	0.81	0.92	1.06	1.22	1.39	1.58	1.80	2.03	2.30
0.70	0.26	0.28	0.32	0.37	0.43	0.51	0.59	0.69	0.79	0.91	1.05	1.19	1.36	1.54	1.74	1.97
0.75	0.22	0.24	0.28	0.32	0.37	0.44	0.50	0.59	0.67	0.78	0.90	1.02	1.16	1.32	1.49	1.69
0.80	0.20	0.21	0.24	0.28	0.32	0.38	0.44	0.52	0.59	0.68	0.78	0.89	1.01	1.15	1.30	1.47
0.85	0.17	0.18	0.21	0.24	0.28	0.33	0.38	0.45	0.52	0.60	0.69	0.78	0.89	1.01	1.14	1.29
0.90	0.15	0.16	0.19	0.21	0.25	0.29	0.34	0.40	0.45	0.53	0.61	0.69	0.78	0.89	1.01	1.14
0.95	0.13	0.14	0.16	0.19	0.22	0.26	0.30	0.35	0.40	0.46	0.53	0.60	0.69	0.78	0.88	1.00
1.00	0.12	0.13	0.15	0.17	0.20	0.23	0.27	0.32	0.36	0.41	0.48	0.54	0.62	0.70	0.79	0.90
1.10	0.10	0.10	0.12	0.13	0.16	0.18	0.21	0.25	0.29	0.33	0.38	0.43	0.49	0.56	0.63	0.72
1.20	0.08	0.08	0.10	0.11	0.13	0.15	0.17	0.21	0.23	0.27	0.31	0.35	0.40	0.46	0.52	0.59
1.30	0.06	0.07	0.08	0.09	0.11	0.13	0.14	0.17	0.19	0.22	0.26	0.29	0.33	0.38	0.43	0.49
1.40	0.05	0.06	0.07	0.08	0.09	0.11	0.12	0.14	0.16	0.19	0.22	0.25	0.28	0.32	0.36	0.41
1.50	0.05	0.05	0.06	0.06	0.08	0.09	0.10	0.12	0.14	0.16	0.18	0.21	0.24	0.27	0.31	0.35
1.60	0.04	0.04	0.05	0.06	0.06	0.08	0.09	0.10	0.12	0.14	0.16	0.18	0.20	0.23	0.26	0.29
1.70	0.03	0.04	0.04	0.05	0.06	0.07	0.08	0.09	0.10	0.12	0.14	0.15	0.18	0.20	0.23	0.26
1.80	0.03	0.03	0.04	0.04	0.05	0.06	0.06	0.08	0.09	0.10	0.12	0.13	0.15	0.17	0.19	0.22
1.90	0.03	0.03	0.03	0.04	0.04	0.05	0.06	0.07	0.08	0.09	0.10	0.12	0.13	0.15	0.17	0.19
2.00	0.02	0.02	0.03	0.03	0.04	0.04	0.05	0.06	0.07	0.08	0.09	0.10	0.11	0.13	0.15	0.17
2.20	0.02	0.02	0.02	0.02	0.03	0.03	0.04	0.05	0.05	0.06	0.07	0.08	0.09	0.10	0.11	0.13
2.50	0.01	0.01	0.01	0.02	0.02	0.02	0.03	0.03	0.04	0.04	0.05	0.05	0.06	0.07	0.08	0.09
3.00	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.03	0.03	0.03	0.04	0.04	0.05	0.06	0.06

AFP (MOM)	hCG(MOM) 1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.5	4.0
0.35	8.95	10.1	11.2	12.5	13.9	15.4	17.0	18.7	20.6	22.7	24.9	27.2	29.7	32.4	41.6	61.4
0.40	6.92	7.79	8.66	9.68	10.7	11.9	13.1	14.5	15.9	17.5	19.2	21.0	22.9	25.0	32.1	47.4
0.45	5.49	6.18	6.87	7.68	8.52	9.45	10.4	11.5	12.6	13.9	15.2	16.7	18.2	19.9	25.5	37.7
0.50	4.43	4.99	5.55	6.20	6.88	7.63	8.40	9.27	10.2	11.2	12.3	13.5	14.7	16.0	20.6	30.4
0.55	3.65	4.11	4.56	5.10	5.66	6.27	6.91	7.62	8.39	9.23	10.1	11.1	12.1	13.2	16.9	25.0
0.60	3.05	3.43	3.81	4.26	4.73	5.24	5.77	6.37	7.01	7.71	8.46	9.24	10.1	11.0	14.1	20.9
0.65	2.57	2.90	3.22	3.60	4.00	4.43	4.88	5.38	5.92	6.52	7.15	7.81	8.53	9.31	12.0	17.7
0.70	2.20	2.48	2.76	3.08	3.42	3.79	4.17	4.60	5.07	5.57	6.11	6.68	7.30	7.96	10.2	15.1
0.75	1.89	2.13	2.36	2.64	2.93	3.25	3.58	3.95	4.34	4.78	5.24	5.72	6.26	6.82	8.76	13.0
0.80	1.64	1.85	2.06	2.30	2.56	2.83	3.12	3.44	3.78	4.16	5.57	4.99	5.45	5.95	7.64	11.3
0.85	1.44	1.63	1.81	2.02	2.24	2.48	2.74	3.02	3.32	3.66	4.01	4.38	4.79	5.22	6.71	9.91
0.90	1.27	1.43	1.59	1.78	1.98	2.19	2.41	2.93	2.66	3.22	3.53	3.86	4.22	4.60	5.91	8.73
0.95	1.12	1.26	1.40	1.56	1.73	1.92	2.11	2.33	2.57	2.82	3.10	3.38	3.70	4.03	5.18	7.65
1.00	1.00	1.13	1.25	1.40	1.55	1.72	1.90	2.09	2.30	2.53	2.78	3.04	3.32	3.62	4.65	6.87
1.10	0.80	0.90	1.00	1.12	1.24	1.38	1.52	1.67	1.84	2.03	2.22	2.43	2.65	2.90	3.72	5.49
1.20	0.66	0.74	0.82	0.92	1.02	1.13	1.25	1.38	1.51	1.67	1.83	2.00	2.18	2.38	3.05	4.51
1.30	0.54	0.61	0.68	0.76	0.84	0.93	1.03	1.14	1.25	1.38	1.51	1.65	1.80	1.96	2.52	3.73
1.40	0.46	0.52	0.57	0.64	0.71	0.79	0.87	0.96	1.05	1.16	1.27	1.39	1.52	1.65	2.12	3.14
1.50	0.39	0.43	0.48	0.54	0.60	0.66	0.73	0.81	0.89	0.98	1.07	1.17	1.28	1.40	1.79	2.65
1.60	0.33	0.37	0.41	0.46	0.51	0.57	0.62	0.69	0.76	0.83	0.91	1.00	1.09	1.19	1.53	2.26
1.70	0.29	0.32	0.36	0.40	0.44	0.49	0.54	0.60	0.66	0.72	0.79	0.87	0.95	1.03	1.32	1.96
1.80	0.24	0.27	0.30	0.34	0.38	0.42	0.46	0.51	0.56	0.62	0.67	0.74	0.81	0.88	1.13	1.67
1.90	0.21	0.24	0.27	0.30	0.33	0.37	0.41	0.45	0.49	0.54	0.60	0.65	0.71	0.78	1.00	1.47
2.00	0.19	0.21	0.23	0.26	0.29	0.32	0.35	0.39	0.43	0.47	0.52	0.56	0.62	0.67	0.86	1.27
2.20	0.14	0.16	0.18	0.20	0.22	0.25	0.27	0.30	0.33	0.36	0.40	0.43	0.47	0.52	0.66	0.98
2.50	0.10	0.11	0.13	0.14	0.16	0.17	0.19	0.21	0.23	0.25	0.28	0.30	0.33	0.36	0.46	0.69
3.00	0.07	0.08	0.09	0.10	0.11	0.12	0.14	0.15	0.16	0.18	0.20	0.22	0.24	0.26	0.33	0.49

5.2.4.3.2 THE EFFECT OF CORRELATION ON THE CALCULATION OF A COMBINED AFP/hCG RISK FACTOR

Likelihood ratios can be calculated for two variables by a combined formula which allows for the correlation between the two, as described in Section 4.2.8. Table 5-32 shows a matrix of likelihood ratios calculated by this second method for a range of hCG and AFP levels. Overall AFP/hCG/age risks can be calculated as described in the preceding section.

5.2.4.3.3 hCG (MOM)/AFP (MOM) RATIOS

Dividing the hCG level (in MOM) by the AFP level (in MOM) produces a ratio which further separates the distributions of Down's syndrome and control samples (Figure 5-24). The median ratio of the Down's syndrome samples was 2.82 compared with a median ratio of 1.01 for the control samples ($p<0.001$ by t-test on unpaired variables). Figure 5-25 shows a probability plot of the hCG/AFP ratios, on a log scale, for the Down's syndrome samples and the controls. The controls form a straight line, showing that they fit a log Gaussian distribution. The Down's samples show a small deviation from the straight line at the ends of the distribution, but applying the Kolmogorov-Smirnov test to these values indicates that this distribution does not deviate significantly from a log Gaussian distribution ($D=0.0947$, $p>0.2$) although the number of cases used (49) is small. The means and standard deviations of the distributions of the ratios were estimated as described in Section 4.2.3.1. The log mean and standard deviation of the control ratios

Table 5 - 32

Combined likelihood ratios for Down's syndrome for AFP and hCG, allowing for the correlation between variables.

AFP (MOM)	hCG (MOM)															
	0·3	0·4	0·5	0·6	0·7	0·8	0·9	1·0	1·1	1·2	1·3	1·4	1·5	1·6	1·7	1·8
0·35	1·04	1·22	1·46	1·76	2·12	2·54	3·04	3·61	4·27	5·01	5·85	6·80	7·86	9·04	10·4	11·8
0·40	0·78	0·90	1·08	1·30	1·56	1·88	2·24	2·66	3·14	3·69	4·30	5·00	5·77	6·64	7·60	8·67
0·45	0·59	0·69	0·82	0·99	1·19	1·43	1·70	2·02	2·39	2·80	3·26	3·79	4·38	5·03	5·76	6·57
0·50	0·47	0·54	0·64	0·77	0·93	1·11	1·33	1·57	1·86	2·17	2·54	2·94	3·40	3·91	4·47	5·10
0·55	0·37	0·43	0·51	0·62	0·74	0·89	1·06	1·25	1·47	1·73	2·01	2·34	2·70	3·10	3·54	4·04
0·60	0·30	0·35	0·42	0·50	0·60	0·72	0·85	1·01	1·19	1·40	1·63	1·88	2·17	2·50	2·86	3·25
0·65	0·25	0·29	0·34	0·41	0·49	0·59	0·70	0·83	0·98	1·14	1·33	1·54	1·78	2·04	2·34	2·66
0·70	0·21	0·24	0·28	0·34	0·41	0·49	0·58	0·69	0·81	0·95	1·20	1·28	1·48	1·69	1·94	2·21
0·75	0·18	0·20	0·24	0·29	0·34	0·41	0·50	0·58	0·68	0·80	0·93	1·07	1·24	1·42	1·62	1·85
0·80	0·15	0·17	0·20	0·24	0·29	0·35	0·41	0·49	0·58	0·67	0·79	0·91	1·05	1·20	1·37	1·56
0·85	0·13	0·15	0·17	0·21	0·25	0·30	0·35	0·42	0·49	0·58	0·67	0·78	0·89	1·03	1·17	1·33
0·90	0·11	0·13	0·15	0·18	0·22	0·26	0·31	0·36	0·42	0·50	0·58	0·67	0·77	0·88	1·01	1·15
0·95	0·10	0·11	0·13	0·16	0·19	0·22	0·27	0·31	0·37	0·43	0·50	0·58	0·67	0·77	0·87	0·99
1·00	0·08	0·10	0·11	0·14	0·16	0·20	0·23	0·27	0·32	0·38	0·44	0·51	0·58	0·67	0·76	0·87
1·10	0·07	0·08	0·09	0·11	0·13	0·15	0·18	0·21	0·25	0·29	0·34	0·39	0·45	0·52	0·59	0·67
1·20	0·05	0·06	0·07	0·08	0·10	0·12	0·14	0·17	0·20	0·23	0·27	0·31	0·36	0·41	0·46	0·53
1·30	0·04	0·05	0·06	0·07	0·08	0·10	0·11	0·13	0·16	0·18	0·21	0·25	0·28	0·33	0·37	0·42
1·40	0·04	0·04	0·05	0·06	0·07	0·08	0·09	0·11	0·13	0·15	0·17	0·21	0·23	0·27	0·30	0·34
1·50	0·03	0·03	0·04	0·05	0·05	0·07	0·08	0·09	0·11	0·12	0·14	0·17	0·19	0·22	0·25	0·28
1·60	0·02	0·03	0·03	0·04	0·05	0·05	0·06	0·08	0·09	0·10	0·12	0·14	0·16	0·18	0·21	0·24
1·70	0·02	0·02	0·03	0·03	0·04	0·05	0·05	0·06	0·07	0·09	0·10	0·12	0·13	0·15	0·17	0·20
1·80	0·02	0·02	0·02	0·02	0·03	0·04	0·05	0·05	0·06	0·07	0·09	0·10	0·11	0·13	0·15	0·17
1·90	0·02	0·02	0·02	0·02	0·03	0·03	0·04	0·05	0·05	0·06	0·07	0·08	0·10	0·11	0·13	0·14
2·00	0·01	0·01	0·02	0·02	0·02	0·03	0·03	0·04	0·05	0·05	0·06	0·07	0·08	0·10	0·11	0·12
2·20	0·01	0·01	0·01	0·02	0·02	0·02	0·03	0·03	0·04	0·04	0·05	0·06	0·06	0·07	0·08	0·09
2·50	0·01	0·01	0·01	0·01	0·01	0·02	0·02	0·02	0·02	0·03	0·03	0·04	0·04	0·05	0·06	0·06
3·00	0·01	0·01	0·01	0·01	0·01	0·01	0·01	0·01	0·01	0·02	0·02	0·02	0·02	0·03	0·03	0·04

AFP (MOM)	hCG (MOM)															
	1·9	2·0	2·1	2·2	2·3	2·4	2·5	2·6	2·7	2·8	2·9	3·0	3·1	3·2	3·5	4·0
0·35	13·4	15·1	17·2	19·3	21·7	24·3	27·1	30·1	33·4	37·0	40·9	45·1	49·6	54·5	71·4	108
0·40	9·86	11·2	12·6	14·2	15·9	17·8	19·8	22·0	24·5	27·1	29·9	32·0	36·3	39·8	52·2	79·3
0·45	7·46	8·44	9·52	10·7	12·0	13·4	15·0	16·6	18·5	20·4	22·6	24·9	27·4	30·0	39·3	59·8
0·50	5·79	6·55	7·38	8·30	9·30	10·4	11·6	12·9	14·3	15·8	17·5	19·3	21·2	23·2	30·4	46·2
0·55	4·58	5·18	5·84	6·57	7·36	8·23	9·17	10·2	11·3	12·5	13·8	15·1	16·7	18·4	24·0	36·4
0·60	3·69	4·18	4·71	5·29	5·93	6·62	7·38	8·20	9·09	10·1	11·1	12·2	13·5	14·8	19·3	29·3
0·65	3·02	3·42	3·85	4·32	4·84	5·41	6·03	6·70	7·43	8·21	9·07	9·99	11·0	12·1	15·8	23·9
0·70	2·50	2·83	3·19	3·58	4·01	4·48	4·99	5·54	6·14	6·79	7·50	8·26	9·08	9·96	13·0	19·7
0·75	2·10	2·37	2·67	3·00	3·36	3·75	4·17	4·64	5·14	5·68	6·27	6·91	7·59	8·33	10·9	16·5
0·80	1·77	2·00	2·26	2·53	2·84	3·17	3·53	3·92	4·34	4·80	5·30	5·83	6·41	7·03	9·18	13·9
0·85	1·51	1·71	1·93	2·16	2·42	2·70	3·01	3·40	3·70	4·09	4·51	4·97	5·46	5·99	7·82	11·8
0·90	1·30	1·47	1·66	1·86	2·08	2·32	2·58	2·87	3·18	3·51	3·88	4·27	4·69	5·14	6·71	10·2
0·95	1·13	1·27	1·43	1·61	1·80	2·01	2·24	2·48	2·75	3·04	3·35	3·69	4·06	4·45	5·80	8·77
1·00	0·98	1·11	1·25	1·40	1·57	1·75	1·95	2·16	2·40	2·65	2·92	3·21	3·53	3·87	5·05	7·63
1·10	0·76	0·86	0·96	1·08	1·21	1·35	1·50	1·67	1·85	2·04	2·25	2·48	2·72	2·98	3·89	5·87
1·20	0·60	0·68	0·76	0·85	0·95	1·06	1·18	1·31	1·45	1·61	1·77	1·95	2·14	2·34	3·06	4·61
1·30	0·48	0·54	0·61	0·68	0·76	0·85	0·95	1·05	1·16	1·28	1·42	1·56	1·71	1·87	2·44	3·68
1·40	0·39	0·44	0·49	0·55	0·62	0·69	0·77	0·85	0·94	1·04	1·15	1·26	1·39	1·52	1·98	2·98
1·50	0·32	0·36	0·41	0·46	0·51	0·57	0·63	0·70	0·78	0·86	0·94	1·04	1·14	1·25	1·62	2·45
1·60	0·27	0·30	0·34	0·38	0·42	0·47	0·52	0·58	0·64	0·71	0·78	0·86	0·95	1·04	1·35	2·03
1·70	0·22	0·25	0·28	0·32	0·36	0·40	0·44	0·49	0·54	0·60	0·66	0·72	0·79	0·87	1·13	1·70
1·80	0·19	0·21	0·24	0·27	0·30	0·34	0·37	0·41	0·46	0·51	0·56	0·61	0·67	0·74	0·96	1·44
1·90	0·16	0·18	0·21	0·23	0·26	0·29	0·32	0·35	0·39	0·43	0·47	0·52	0·57	0·63	0·82	1·23
2·00	0·14	0·16	0·18	0·20	0·22	0·25	0·27	0·30	0·34	0·37	0·41	0·45	0·49	0·54	0·70	1·05
2·20	0·11	0·12	0·13	0·15	0·17	0·19	0·21	0·23	0·25	0·28	0·31	0·34	0·37	0·40	0·53	0·79
2·50	0·07	0·08	0·09	0·10	0·11	0·13	0·14	0·16	0·17	0·19	0·21	0·23	0·25	0·27	0·36	0·54
3·00	0·04	0·05	0·05	0·06	0·06	0·07	0·08	0·09	0·10	0·11	0·12	0·13	0·14	0·16	0·20	0·30

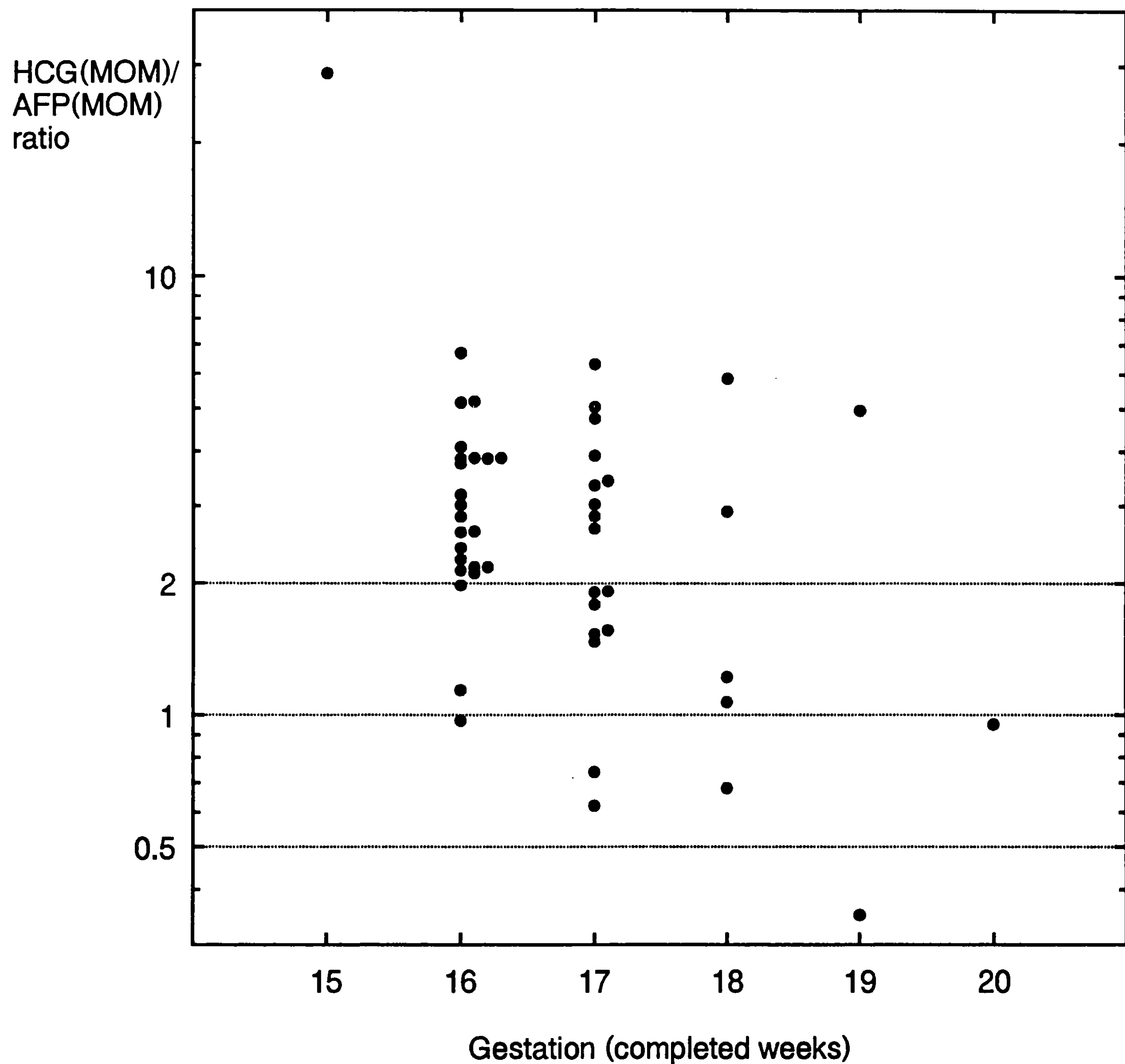
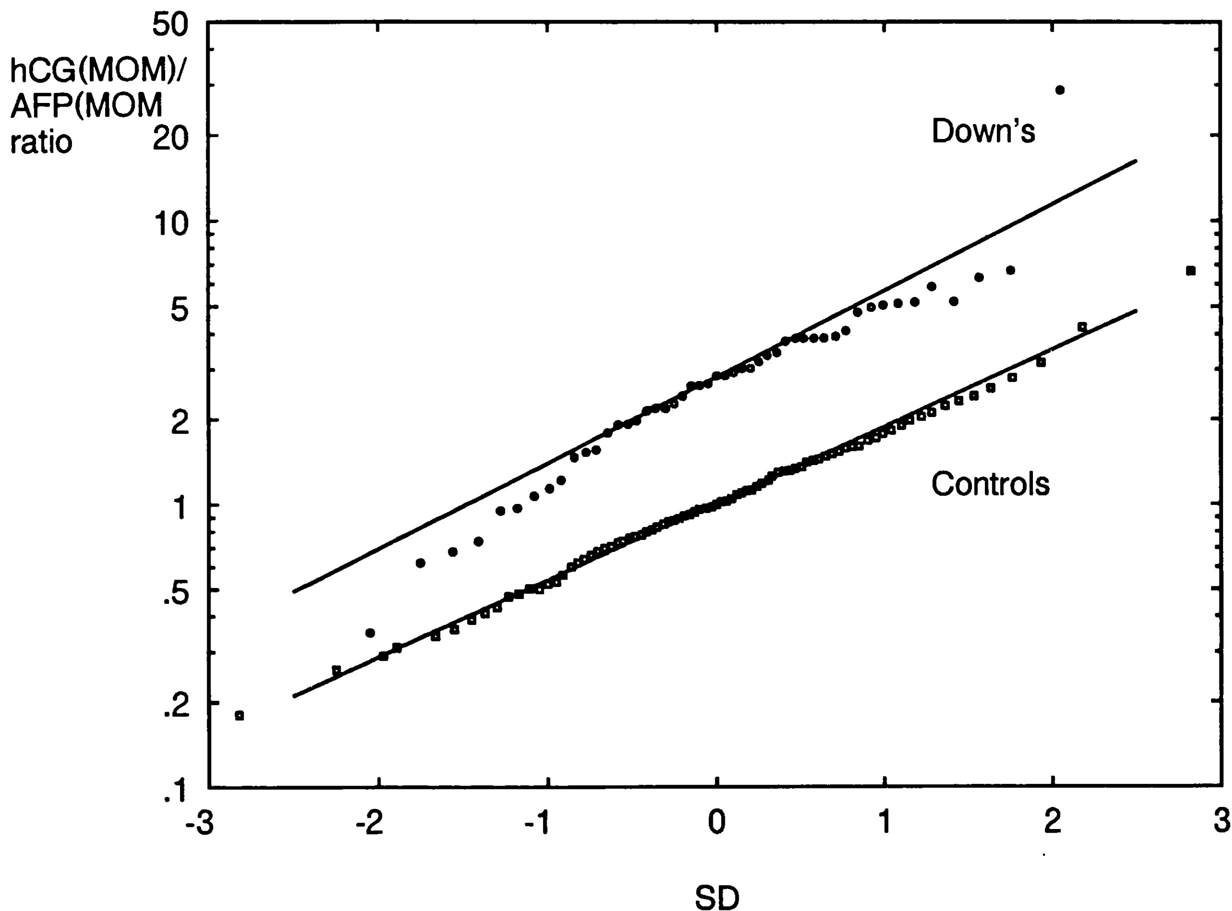


Figure 5 - 24

Ratios of hCG(MOM)/AFP(MOM) in maternal serum in 49 Down's syndrome pregnancies compared with the levels in 410 unaffected controls.



<u>Figure 5 - 25</u>

Probability plot of hCG(MOM)/AFP(MOM) ratios, on a log scale for Down's syndrome and selected control pregnancies. The continuous lines are those defined by log Gaussian distributions with means and standard deviations as given in the text (Section 5.2.4.3.3).

are 0.0043 and 0.2714 respectively and for the Down's syndrome ratios 0.4502 and 0.3046 respectively. The log Gaussian distributions of the control and Down's samples are shown in Figure 5-26. The two curves have an intersection point at a ratio of 1.72. The observed numbers and proportions of Down's syndrome and unaffected pregnancies with ratios greater than or equal to specific values are shown in Table 5-33 indicating, for example, that 57% detection of Down's syndrome pregnancies could be achieved using hCG/AFP ratios at a 5.9% false positive rate.

The hCG/AFP ratio can be treated as a new variable and risks calculated from the overlapping log Gaussian distributions of these ratios for Down's syndrome and unaffected pregnancies as described in Section 4.2.8. The combined risk of Down's syndrome for a given age and hCG/AFP ratio is estimated by multiplying the left hand side of the age specific risk by the likelihood ratio. An individual woman's risk of having a Down's syndrome fetus between 15-20 weeks gestation, based on the age and serum AFP and hCG levels is shown in Table 5-34.

5.2.4.3.4 PREDICTED DETECTION RATES

Table 5-35 shows the predicted detection rates in the west of Scotland pregnant population at a 5% false positive rate for each of the three methods described above for deriving risks from AFP, hCG and maternal age, and also gives the predicted false positive rates at a 60% detection rate. The detection rates at a 5% false positive rate are similar for the various methods (56-57%)

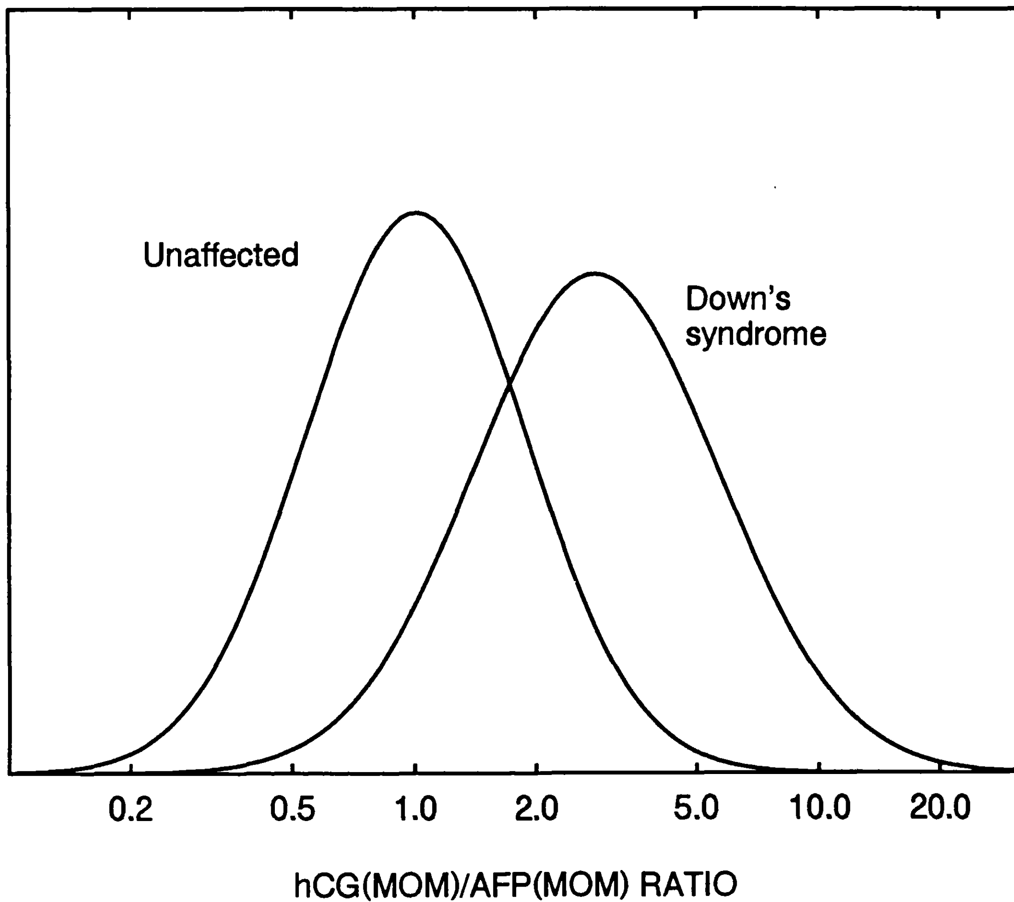


Figure 5 - 26

Log Gaussian distributions of hCG(MOM)/AFP(MOM) ratios in Down's syndrome pregnancies and unaffected control pregnancies. These are plotted from the means and standard deviations of 49 Down's syndrome and 410 unaffected pregnancies as given in Section 5.2.4.3.3.

Table 5 -33

Detection rates for Down's syndrome and corresponding false positive rates at selected hCG/AFP ratios.

hCG/AFP ratio	% (n) of Down's syndrome pregnancies ≥the ratio (n=49)	% (n) of control pregnancies ≥ the ratio (n=410)
≥5.0	16 (8)	1.0 (4)
≥4.5	20 (10)	1.0 (4)
≥4.0	22 (11)	1.5 (6)
≥3.5	35 (17)	2.0 (8)
≥3.0	45 (22)	3.4 (14)
≥2.5	57 (28)	5.9 (24)
≥2.0	67 (33)	11 (49)
≥1.5	80 (39)	25 (102)
≥1.0	88 (43)	51 (209)
≥0.5	98 (48)	87 (356)

Table 5 - 34

Risk estimates of finding a Down's syndrome fetus at mid-trimester, based on maternal age and hCG/AFP ratio at 15-20 weeks gestation.

Maternal age (years)	Human chorionic gonadotrophin (MOM)/alpha-fetoprotein (MOM) ratio															
	5·0	4·5	4·0	3·5	3·2	3·1	3·0	2·9	2·8	2·7	2·6	2·5	2·4	2·3	2·2	2·1
15	1:75	1:100	1:140	1:210	1:260	1:290	1:310	1:360	1:370	1:410	1:450	1:500	1:560	1:620	1:690	1:780
16	1:74	1:100	1:140	1:200	1:260	1:280	1:310	1:340	1:370	1:410	1:450	1:500	1:560	1:620	1:690	1:780
17	1:74	1:100	1:140	1:200	1:260	1:280	1:310	1:340	1:370	1:410	1:450	1:500	1:560	1:620	1:690	1:770
18	1:74	1:100	1:140	1:200	1:260	1:280	1:310	1:360	1:370	1:410	1:450	1:500	1:550	1:620	1:690	1:770
19	1:73	1:100	1:140	1:200	1:260	1:280	1:300	1:330	1:370	1:400	1:440	1:490	1:550	1:610	1:680	1:760
20	1:73	1:100	1:140	1:200	1:250	1:280	1:300	1:330	1:360	1:400	1:440	1:490	1:540	1:600	1:670	1:750
21	1:71	1:100	1:140	1:200	1:250	1:270	1:300	1:330	1:360	1:390	1:430	1:480	1:530	1:590	1:660	1:740
22	1:70	1:95	1:130	1:190	1:250	1:270	1:290	1:320	1:350	1:390	1:430	1:470	1:520	1:580	1:650	1:730
23	1:69	1:93	1:130	1:190	1:240	1:260	1:290	1:310	1:340	1:380	1:420	1:460	1:510	1:570	1:640	1:710
24	1:66	1:90	1:130	1:180	1:230	1:250	1:280	1:300	1:330	1:370	1:400	1:450	1:500	1:550	1:620	1:690
25	1:64	1:87	1:120	1:180	1:220	1:240	1:270	1:290	1:320	1:350	1:390	1:430	1:480	1:530	1:590	1:660
26	1:61	1:83	1:120	1:170	1:210	1:230	1:250	1:280	1:300	1:340	1:370	1:410	1:460	1:500	1:570	1:640
27	1:59	1:80	1:110	1:160	1:210	1:230	1:250	1:270	1:300	1:330	1:360	1:400	1:440	1:490	1:550	1:610
28	1:53	1:72	1:100	1:150	1:190	1:200	1:220	1:240	1:270	1:290	1:320	1:360	1:400	1:440	1:490	1:550
29	1:48	1:65	1:91	1:130	1:170	1:180	1:200	1:220	1:240	1:270	1:290	1:320	1:360	1:400	1:450	1:500
30	1:43	1:58	1:82	1:120	1:150	1:160	1:180	1:200	1:220	1:240	1:260	1:290	1:320	1:360	1:400	1:450
31	1:38	1:51	1:72	1:100	1:130	1:140	1:160	1:170	1:190	1:210	1:230	1:250	1:280	1:310	1:350	1:390
32	1:32	1:44	1:61	1:89	1:110	1:120	1:130	1:150	1:160	1:180	1:200	1:220	1:240	1:270	1:300	1:340
33	1:27	1:37	1:52	1:75	1:95	1:100	1:110	1:120	1:160	1:150	1:170	1:180	1:200	1:230	1:250	1:280
34	1:22	1:30	1:43	1:62	1:79	1:86	1:94	1:100	1:110	1:120	1:140	1:150	1:170	1:190	1:210	1:230
35	1:18	1:25	1:34	1:50	1:64	1:69	1:76	1:83	1:91	1:100	1:110	1:120	1:140	1:150	1:170	1:190
36	1:15	1:20	1:28	1:40	1:51	1:56	1:61	1:66	1:73	1:80	1:88	1:100	1:110	1:120	1:140	1:150
37	1:11	1:16	1:22	1:32	1:40	1:44	1:48	1:52	1:58	1:63	1:70	1:77	1:86	1:100	1:110	1:120
38		1:12	1:17	1:25	1:31	1:34	1:37	1:41	1:45	1:49	1:54	1:60	1:67	1:74	1:83	1:93
39			1:13	1:19	1:24	1:26	1:29	1:32	1:35	1:38	1:42	1:47	1:52	1:58	1:64	1:72
40			1:10	1:15	1:19	1:20	1:22	1:24	1:27	1:29	1:32	1:36	1:40	1:44	1:49	1:56
41				1:11	1:14	1:15	1:17	1:18	1:20	1:22	1:24	1:27	1:30	1:33	1:37	1:42
42			>1:10		1:11	1:12	1:13	1:14	1:15	1:17	1:19	1:21	1:23	1:26	1:29	1:32
43							1:10	1:11	1:12	1:13	1:14	1:16	1:17	1:19	1:21	1:24
44										1:10	1:11	1:12	1:13	1:15	1:16	1:19
45														1:11	1:12	1:14

Maternal age (years)	Human chorionic gonadotrophin (MOM)/alpha-fetoprotein (MOM) ratio															
	2·0	1·9	1·8	1·7	1·6	1·5	1·4	1·3	1·2	1·1	1·0	0·9	0·8	0·7	0·6	0·5
15	1:880	1:990	1:1100	1:1300	1:1500	1:1800	1:2000	1:2400	1:2900	1:3400						
16	1:880	1:990	1:1100	1:1300	1:1500	1:1700	1:2000	1:2400	1:2900	1:3400						
17	1:880	1:990	1:1100	1:1300	1:1500	1:1700	1:2000	1:2400	1:2800	1:3400						
18	1:870	1:980	1:1100	1:1300	1:1500	1:1700	1:2000	1:2300	1:2800	1:3400						
19	1:860	1:970	1:1100	1:1300	1:1500	1:1700	1:2000	1:2300	1:2800	1:3300						
20	1:850	1:960	1:1100	1:1300	1:1500	1:1700	1:2000	1:2300	1:2800	1:3300				<1:4000		
21	1:840	1:950	1:1100	1:1200	1:1400	1:1700	1:1900	1:2300	1:2700	1:3300	1:4000					
22	1:820	1:930	1:1100	1:1200	1:1400	1:1600	1:1900	1:2200	1:2700	1:3200	1:4000					
23	1:810	1:910	1:1000	1:1200	1:1400	1:1600	1:1900	1:2200	1:2600	1:3100	1:3900					
24	1:790	1:880	1:1000	1:1200	1:1300	1:1500	1:1800	1:2100	1:2600	1:3000	1:3700					
25	1:760	1:850	1:970	1:1100	1:1300	1:1500	1:1700	1:2000	1:2500	1:2900	1:3600					
26	1:720	1:810	1:930	1:1100	1:1300	1:1400	1:1700	1:1900	1:2300	1:2800	1:3400					
27	1:700	1:780	1:900	1:1000	1:1200	1:1400	1:1600	1:1900	1:2300	1:2700	1:3300					
28	1:630	1:700	1:810	1:920	1:1100	1:1200	1:1400	1:1700	1:2000	1:2400	1:3000	1:3800				
29	1:570	1:640	1:730	1:840	1:970	1:1100	1:1300	1:1500	1:1900	1:2200	1:2700	1:3400				
30	1:510	1:570	1:650	1:750	1:870	1:1000	1:1200	1:1400	1:1700	1:2000	1:2400	1:3000	1:3800			
31	1:450	1:500	1:570	1:660	1:760	1:870	1:1000	1:1200	1:1400	1:1700	1:2100	1:2700	1:3400			
32	1:380	1:430	1:490	1:560	1:650	1:750	1:880	1:1000	1:1200	1:1500	1:1800	1:2300	1:2900	1:3600		
33	1:320	1:360	1:410	1:470	1:550	1:630	1:740	1:870	1:1000	1:1200	1:1500	1:1900	1:2400	1:3100		
34	1:270	1:300	1:340	1:390	1:450	1:520	1:610	1:720	1:860	1:1000	1:1300	1:1600	1:2000	1:2500	1:3400	
35	1:210	1:260	1:280	1:320	1:370	1:420	1:500	1:580	1:700	1:830	1:1000	1:1300	1:1600	1:2000	1:2800	1:3800
36	1:170	1:190	1:220	1:250	1:290	1:340	1:400	1:460	1:560	1:660	1:820	1:1000	1:1300	1:1600	1:2200	1:3100
37	1:140	1:150	1:170	1:200	1:230	1:270	1:310	1:370	1:440	1:520	1:650	1:800	1:1000	1:1300	1:1800	1:2400
38	1:110	1:120	1:140	1:160	1:180	1:210	1:240	1:280	1:340	1:410	1:500	1:630	1:790	1:1000	1:1400	1:1900
39	1:82	1:92	1:110	1:120	1:140	1:160	1:190	1:220	1:270	1:320	1:390	1:490	1:620	1:780	1:1100	1:1500
40	1:63	1:71	1:81	1:93	1:110	1:123	1:150	1:170	1:200	1:240	1:300	1:380	1:480	1:600	1:820	1:1100
41	1:48	1:54	1:61	1:70	1:81	1:93	1:110	1:130	1:150	1:180	1:230	1:280	1:360	1:450	1:620	1:900
42	1:36	1:41	1:47	1:54	1:62	1:71	1:84	1:100	1:120	1:140	1:170	1:220	1:270	1:350	1:470	1:650
43	1:27	1:31	1:35	1:40	1:46	1:53	1:63	1:74	1:89	1:110	1:130	1:160	1:210	1:260	1:350	1:490
44	1:21	1:24	1:27	1:31	1:36	1:41	1:48	1:57	1:68	1:81	1:100	1:130	1:160	1:200	1:270	1:380
45	1:15	1:17	1:20	1:23	1:26	1:30	1:35	1:42	1:50	1:59	1:73	1:110	1:120	1:150	1:200	1:280

Table 5 - 35

Detection rates for Down's syndrome and corresponding false positive rates predicted by combining maternal age risks with those derived from AFP and hCG levels.

Calculation method	5% false positive rate		60% detection rate	
	Det^{n} rate (%)	Cut-off risk	False positive rate (%)	Cut-off risk
(1) Combined AFP and hCG likelihood ratios (ignoring correlation)	57	1:290	6.1	1:340
(2) Combined AFP and hCG likelihood ratios (including correlation)	56	1:265	6.2	1:320
(3) hCG/AFP ratios	57	1:235	5.9	1:280

although a different risk threshold is required for each method to achieve the 5% follow-up rate. The false positive rates and corresponding detection rates vary for different maternal age groups (Table 5-36) although the differences between the methods used are small. For example, a detection rate of 36-39% at a false positive rate of 2.2-2.7% in the age group 20-24 years, compares with a detection rate of 72-75% at a false positive rate of 15-19% in the 35-37 years age group.

Using hCG/AFP/age screening increases the overall odds of finding an affected pregnancy in the high risk group (Table 5-37) compared with AFP/age screening or maternal age screening (Table 5-8). The overall odds of finding an affected pregnancy in the high risk group (risk ≥1:235) are 1:60 and are reduced to 1:1,500 in the low risk group (risk <1:235). The overall odds of finding an affected pregnancy also vary with different maternal ages. For example in the age group 25-34 the overall odds of finding an affected pregnancy if in the high risk group are 1:70, but reduced to 1:1,500 if in the low risk group.

5.2.4.4 USING hCG IN SCREENING FOR OTHER CHROMOSOME ABNORMALITIES

In prospective practice the use of elevated hCG results will not detect trisomies 18 and 13 and the unbalanced translocations, and different selection criteria are required if such pregnancies are not to be missed. However the increased detection of Down's to 57% using hCG/AFP/age screening represents 50% of all autosomal trisomies present at mid-trimester.

Table 5 - 36

Detection rates for Down's syndrome and corresponding false positive rates for different maternal age groups predicted by combining maternal age risks with those derived from AFP and hCG levels.

	Calculation method					
	(1)		(2)		(3)	
Age group (yrs)	Det^n rate (%)	False pos. rate (%)	Det^n rate (%)	False pos. rate (%)	Det^n rate (%)	False pos. rate (%)
<20	35	2.0	35	2.2	38	2.4
20-24	36	2.2	37	2.6	39	2.7
25-29	48	2.8	41	3.2	45	3.3
30-34	58	6.1	58	6.3	58	6.3
35-37	75	19	73	17	72	15
38-40	85	44	86	38	83	35
>40	96	81	94	70	93	62
Overall	57	5.0	56	5.0	57	5.0

Calculation methods:
(1) Combined AFP and hCG likelihood ratios (ignoring correlation).
(2) Combined AFP and hCG likelihood ratios (including correlation).
(3) hCG/AFP ratios

Table 5 - 37

Comparison of overall odds of a Down's syndrome pregnancy at mid-trimester, using hCG/AFP/age screening with an overall false positive rate of 5%.

	OVERALL ODDS OF AN AFFECTED PREGNANCY
(a) POPULATION RISKS	
Overall	1: 690
Age ≥35 years	1: 150
Age 25-34 years	1: 820
Age 25 years	1:1200
(b) WITH hCG/AFP/AGE RISK ≥1:235	
Overall	1: 60
Age ≥35 years	1: 50
Age 25-34 years	1: 70
Age <25 years	1: 85
(c) WITH hCG/AFP/AGE RISK <1:235	
Overall	1:1500
Age ≥35 years	1: 670
Age 25-34 years	1:1500
Age <25 years	1:1900

A second high risk group, to detect mainly trisomy 18 pregnancies, could potentially be identified using low hCG results. For example, at a cut-off of ≥0.30 MOM, 8 out of 12 (75%) of the trisomy 18 pregnancies, 1 out of 8 (13%) of the unbalanced translocations and 1 out of 9 of the sex chromosome abnormalities (Table 5-14) would be detected, but at a cost of adding 2.7% to the amniocentesis rate. Alternatively calculating the product of the serum AFP and hCG results in MOM would allow selection of a group of pregnancies below a chosen cut-off. For example, an AFP X hCG factor of ≥0.20 would include 8 out of 12 (75%) trisomy 18 and 1 out of 8 (13%) of the unbalanced chromosome abnormalities in this series, but would add 1.2% to the amniocentesis rate.

5.2.4.5 OTHER ANALYTES IN SCREENING FOR DOWN'S SYNDROME

A summary of the predicted detection rates for Down's syndrome at a 5% false positive rate for different analytes, either singly or in combinations of two or three analytes, together with maternal age, is shown in Table 5-38. These were calculated as described in Section 4.2.8 taking into account correlation where significant. AFP is included in all combinations of two or more analytes as it is always likely to be incorporated in screening protocols because of its usefulness in predicting neural tube defects.

Table 5 -38

Predicted detection rates for Down's syndrome at a 5% false positive rate for combinations of one, two and three analytes, together with maternal age.

Analyte combination	Predicted detection rate	95% confidence interval
AFP/age	31	21-41
hCG/age	51	36-66
UE3/age	29	17-43
SP_1/age	34	20-48
Frß-hCG/age	44	35-59
AFP/hCG/age	57	42-71
AFP/UE3/age	33	20-48
AFP/SP_1/age	42	29-58
AFP/Frß-hCG/age	51	43-66
AFP/hCG/UE3/age	53	38-67
AFP/hCG/SP_1/age	57	42-71
AFP/hCG/Frß-hCG/age	55	47-70
AFP/UE3/SP_1/age	41	28-57
AFP/UE3/Frß-hCG/age	49	35-65
AFP/SP_1/Frß-hCG/age	55	40-70

5.2.4.5.1 SP_1

Adding SP_1 to an existing AFP/age screening programme is less effective than adding hCG (42% vs. 57% detection). SP_1 used in addition to AFP and hCG does not increase the detection rate (57% vs. 57% detection). Adding SP_1 improves slightly the predicted detection rate when used in combination with AFP/UE3/age (41% vs. 33% detection) or AFP/Frß-hCG/age (55% vs. 51% detection).

5.2.4.5.2 UE3

Adding UE3 results to the risk estimation and comparing the detection and false positive results with those for the AFP/hCG/age combination shows a loss of detection for Down's syndrome (53% vs. 57% detection) at a 5% false positive rate. This loss of detection when adding UE3 to the AFP/hCG/age combination is found at a range of false positive rates, as can be seen in Figure 5-27, which shows detection rates for Down's syndrome and corresponding false positive rates for age, AFP/age, UE3/AFP/age, hCG/AFP/age and UE3/AFP/hCG/age. False positive rates vary for different maternal age groups, and from this study the addition of UE3 to the AFP/hCG/age combination causes a loss of detection amongst younger women (Table 5-39).

Adding UE3 also decreases the detection rate slightly when added to the AFP/SP_1/age combination (41% vs. 42% detection) and also to AFP/Frß-hCG/age (49% vs. 51% detection).

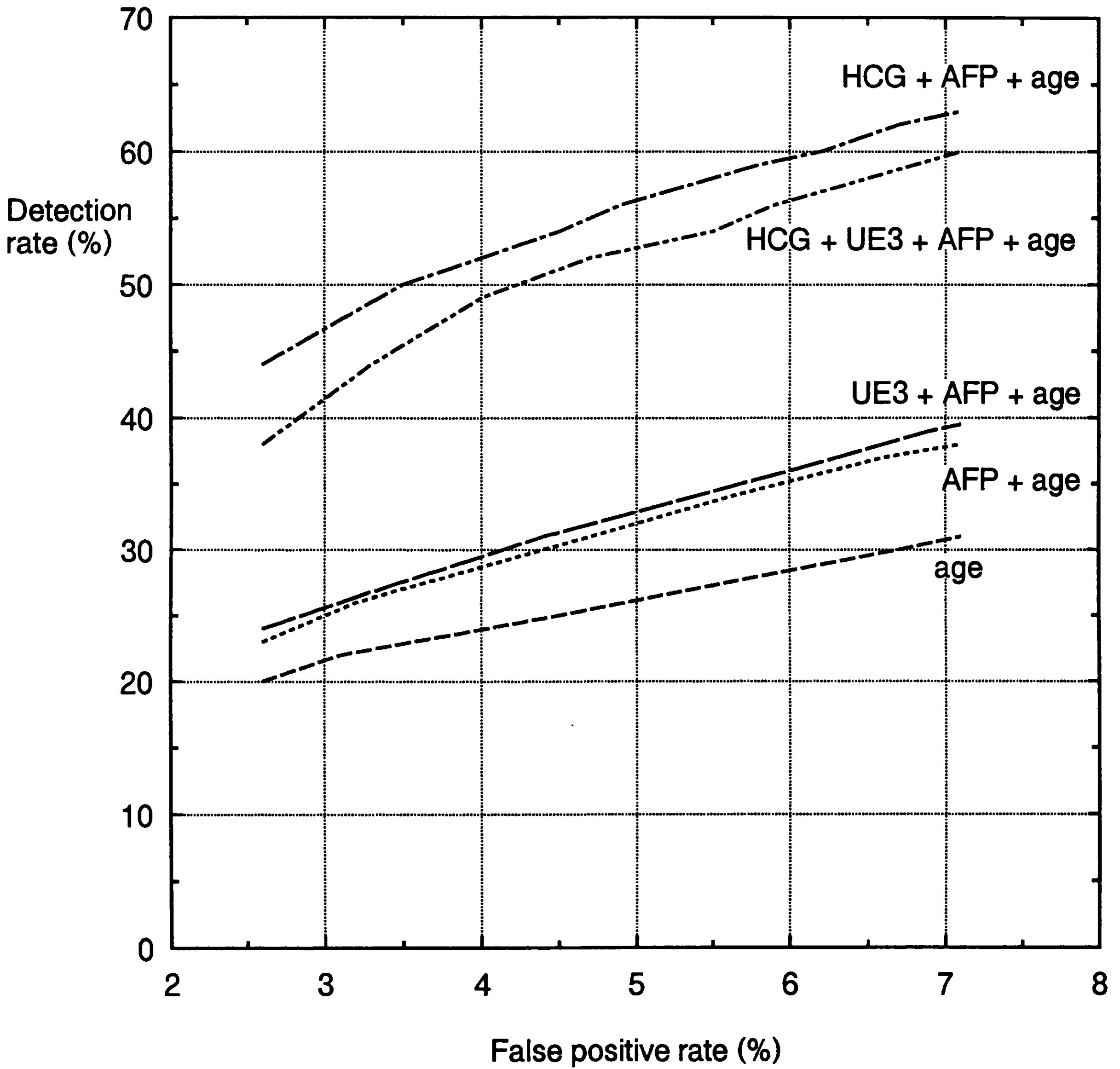


Figure 5 - 27

Detection rates for Down's syndrome and corresponding false positive rates predicted by combining maternal age risks with different combinations of AFP, hCG and UE3.

Table 5 - 39

Comparison of the detection rates for Down's syndrome and corresponding false positive rates for different maternal age groups predicted by combining maternal age risks with risks derived either from AFP/hCG or AFP/hCG/UE3.

	AFP/hCG/age		AFP/hCG/UE3/age	
Age group (yrs)	Det^n rate (%)	False +ve rate (%)	Det^n rate (%)	False +ve rate (%)
<20	35	2.2	33	2.4
20-24	37	2.6	33	2.5
25-29	41	3.2	36	3.1
30-34	58	6.3	52	6.4
35-37	73	17	73	16
38-40	86	38	86	37
>40	94	70	98	74
Overall	56	5.0	53	5.0

5.2.4.5.3 FREE β hCG

From this study adding Frβ-hCG to an existing AFP screening programme is less effective than adding intact hCG (51% vs. 57% detection). Although the median Frβ-hCG level in Down's syndrome is higher than the median for intact hCG, the greater standard deviation, caused by the poorer precision of the Frβ-hCG assay, increases the false positive rate.

PHASE 3

5.3 ROUTINE APPLICATION OF hCG/AFP/AGE SCREENING

5.3.1 PROSPECTIVE TRIAL OF hCG/AFP/AGE SCREENING

In the preceding section, retrospective analysis of the performance of five serum analytes as markers for Down's syndrome has shown that the most effective combination is AFP, intact hCG and maternal age. In order to determine the impact of hCG/AFP/ age screening in routine practice, a blind trial of this type of screening was carried out between July 1989 and June 1990. All samples sent for routine AFP screening from three Glasgow maternity hospitals (The Queen Mother's Hospital, Glasgow Royal Maternity Hospital and Rutherglen Maternity Hospital) were also analysed for intact hCG levels as described in Section 4.1.3.1. The aims of the trial were to determine the detection rates for Down's syndrome and corresponding false positive rates at different risk thresholds, using different methods of calculating risks, and to assess the effect of maternal weight and complications of pregnancy (twins, threatened abortion, insulin dependent diabetes) on analyte levels. The hCG results were not reported but were stored as raw data and not used for risk calculation until the outcomes of the pregnancies were known.

AFP and hCG were analysed in total of 7830 pregnancies, consisting of 7748 singleton pregnancies, 81 sets of twins and one set of triplets. The distribution of the gestations at which the samples were taken is shown in Figure 5-28 and the age distribution of the women in Figure 5-29. The median age was 26.6 years and a comparison with the age distribution of the west of

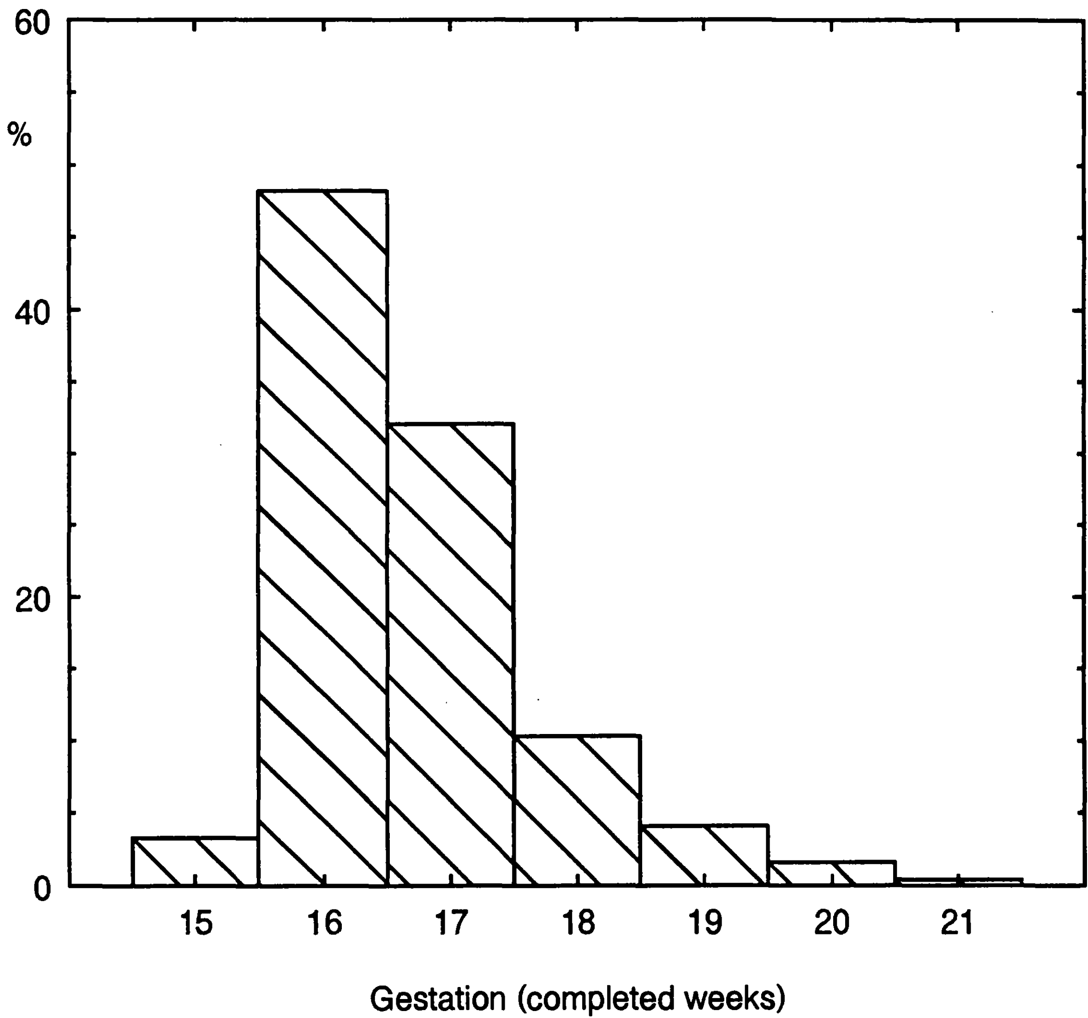
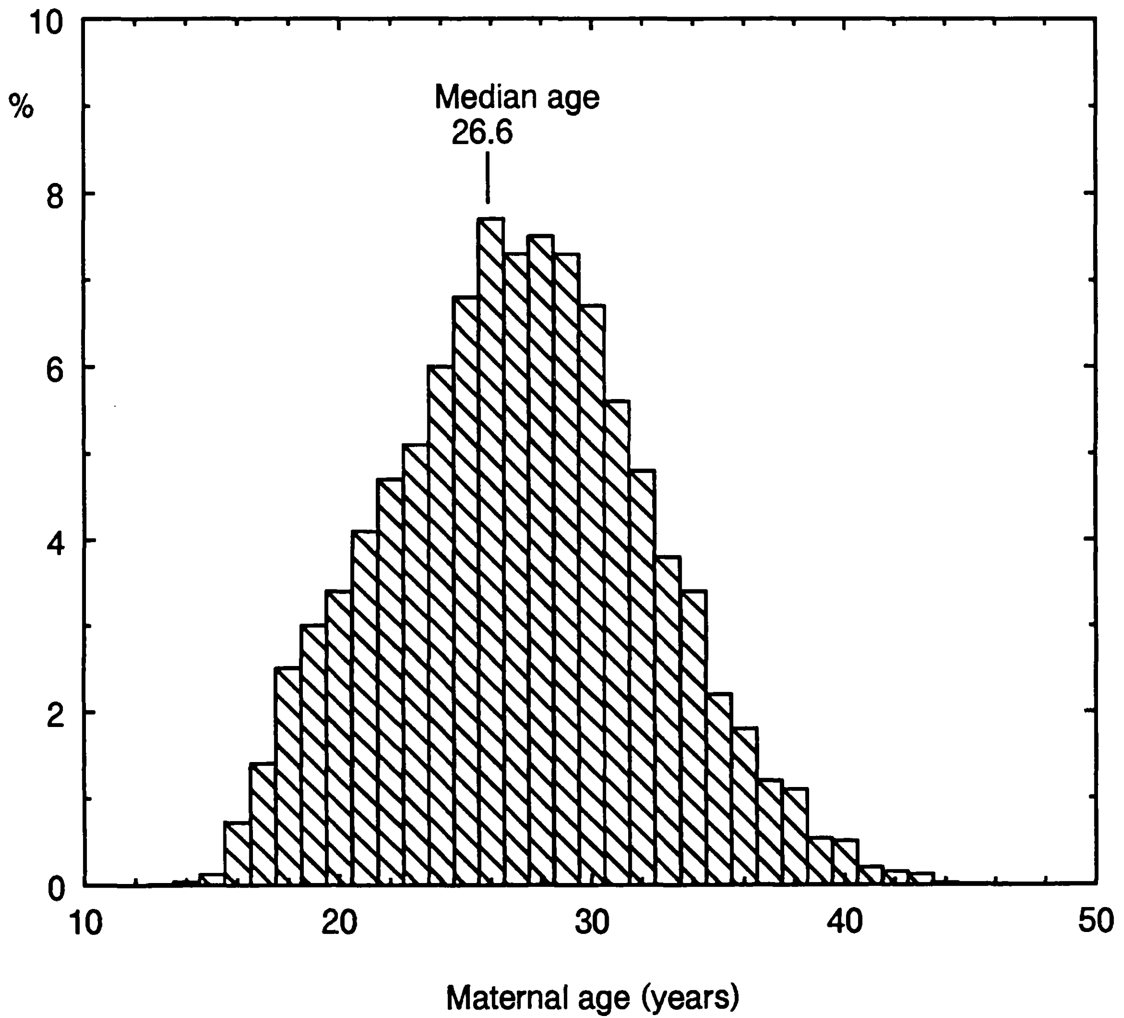


Figure 5 - 28

The distribution of gestations at which samples were taken in the prospective trial of hCG/AFP/age screening.



<u>Figure 5 - 29</u>

The distribution of maternal ages of women whose samples were in the prospective trial of hCG/AFP/age screening.

Scotland pregnant population showed that this distribution was representative of the whole pregnant population.

There were 18 autosomal trisomy pregnancies identified in this group, consisting of 16 Down's syndrome (15 in singleton pregnancies and 1 in a twin pregnancy) and 2 cases of trisomy 18. Table 5-40 compares detection and false positive rates for the samples in this trial with those predicted retrospectively (see Section 5.2.4.3.4). Risks were calculated by the hCG(MOM)/AFP(MOM) ratio method (Section 5.2.4.3.3). At any given risk threshold the detection rate actually achieved is close to that predicted from the retrospective study, but the false positive rate is higher.

To compare the effect of different calculation methods on the detection rate for Down's syndrome, risks were also calculated using the formula which includes correlation (Section 5.2.4.3.2). Table 5-41 gives the detection and false positive rates by this method. Comparing these with the data obtained by the ratio method (Table 5-40) shows that, at equivalent detection rates, the false positive rates obtained using the ratio method are lower, and are generated by lower threshold cut-off risks. For example, a 53% detection rate at a 6.2% false positive rate is obtained using a 1:220 cut-off risk by the ratio method compared with a 53% detection rate at a 6.3% false positive rate using a 1:260 cut-off risk by the formula which includes correlation.

The two trisomy 18 pregnancies had hCG levels of 0.21 MOM and 0.16 MOM and AFP levels of 0.38 MOM and 1.62 MOM respectively. This second case was also affected by spina bifida.

Table 5 - 40

Comparison of detection rates for Down's syndrome and corresponding false positive rates achieved in the prospective trial with those predicted from the retrospective study. Risks calculated by the hCG(MOM)/AFP(MOM) method.

	Prospective (n = 7830)		Retrospective (Controls = 410 Down's = 49)	
Cut-off risk	False +ve rate %	Det^{n} rate Down's (n = 15) % (n)	False +ve rate %	Det^{n} rate Down's %
1:240	6.7	60 (9)	5.1	57
1:220	6.2	53 (8)	4.5	55
1:200	5.6	53 (8)	4.1	54

Table 5 - 41

Effect of calculating risks in the prospective trial by the combined formula including correlation on the false positive rate and detection rate for Down's syndrome.

Cut-off risk	False +ve rate (n=7830) %	Det^{n} rate Down's (n=15) %	(n)
1:280	6.8	60	(9)
1:260	6.3	53	(8)
1:240	5.8	53	(8)

Over time, 12 AFP and hCG results had now been accumulated from 12 trisomy 18 pregnancies. These were used, together with the AFP and hCG results from 7,830 unaffected pregnancies to look at ways of utilizing these results to screen for trisomy 18. Using the hCG results alone, in MOM, 8 out of 12 (67%) of affected pregnancies had values ≤0.25 MOM, compared with 1.1% of unaffected pregnancies. Using the product of AFP and hCG (in MOM), 8 out of 12 (67%) of affected pregnancies (67%) had a factor of ≤0.2, compared with 1.4% of unaffected pregnancies.

5.3.1.1 EFFECT OF MATERNAL WEIGHT ON hCG AND AFP LEVELS

The median hCG and AFP levels on different maternal weight bands are shown in Table 5-42, along with the median likelihood ratio calculated from hCG/AFP ratios. Both AFP and hCG levels are increased in lighter women and are reduced in heavier than average women. The effect is less marked for hCG.

When using the hCG/AFP ratio method to calculate risks, weight correction will make little difference to the 85% of individual women with weights ±15 Kg of the median weight, due to the similar changes in AFP and hCG cancelling each other out. Assay imprecision is likely to have a similar effect. However, for women with weights outside this range, weight correction will provide a more accurate individual risk. However, overall, weight correction will have little effect on the overall screening performance.

Table 5 - 42

The effect of maternal weight on AFP and hCG levels and on the likelihood ratio (LR) derived from these.

Weight (Kg)	n	Median hCG	Median AFP	Median LR from hCG/AFP ratio
<45	100	1.13	1.25	0.21
45.0-54.9	1479	1.10	1.15	0.28
55.0-64.9	2810	1.03	1.00	0.30
65.0-74.9	1499	0.97	0.92	0.32
75.0-84.9	532	0.90	0.84	0.35
≥85.0	301	0.89	0.83	0.39
OVERALL				
59.0	6723	0.97	1.00	0.30

5.3.1.2 THE EFFECT OF TWINS ON hCG LEVELS

The median hCG level in 81 twin pregnancies was 1.85 MOM and the median AFP level 1.91 MOM. Twin pregnancies therefore have approximately double the levels of hCG and AFP found in singleton pregnancies. The one twin pregnancy discordant for Down's syndrome had an hCG level of 4.63 MOM and an AFP level of 1.94 MOM. At this stage accurate Down's syndrome risks cannot be calculated for twin pregnancies due to lack of data. The feasibility of calculating risks for twin pregnancies would require the analysis of a large series of affected twin pregnancies, both discordant and concordant for Down's syndrome.

5.3.1.3 THE EFFECT OF THREATENED ABORTION ON hCG LEVELS

In this series of 7830 pregnancies, 392 had the 'threatened abortion' box ticked on the request form, but in the majority of cases the interval between the event and drawing of the screening blood sample was not known . These samples had a median hCG level of 1.03 MOM and a median AFP level of 1.07 MOM. Episodes of bleeding, particularly in the few weeks preceding the collection of the screening blood sample, may cause a rise in AFP levels due to feto-maternal haemorrhage (Wald *et al.*, 1977, Lidbjork *et al.*, 1977). In a sub-group of 28 pregnancies with AFP levels ≥2.0 MOM, the most likely to have had recent threatened abortion, the median hCG level was 1.09 MOM and the median AFP level 2.55 MOM. The hCG levels are virtually unchanged in this group of patients. There will therefore be a tendency for risks to be underestimated in

cases of threatened abortion.

5.3.1.4 THE EFFECT OF INSULIN DEPENDENT DIABETES ON hCG AND AFP LEVELS

There were 15 cases of insulin dependent diabetes (IDDM) in this series, which had a median AFP value of 0.96 MOM and a median hCG value of 0.90 MOM. A further 41 cases were identified from routine screening between August 1992 and August 1993. The median AFP value of the 56 cases was 0.94 MOM and for hCG was 0.90 MOM. Two of the 56 (3.6%) were in the high risk (≥1:220) group and three of the 56 had (5.4%) AFP values greater than 2.0 MOM. IDDM patients do not appear to be either over- or under- represented in either of the high risk groups.

5.3.2 hCG/AFP/AGE SCREENING IN ROUTINE PROSPECTIVE USE

Following the development of protocols for the use of hCG, AFP and maternal age in a population screening programme and the demonstration that this combination resulted in significant improvement in the sensitivity of detection of Down's syndrome pregnancies (Section 5.3.1) the routine maternal serum screening programme in the west of Scotland was extended to include hCG analysis in September 1991.

Before implementing this change to routine clinical practice substantial re-writing of the existing computerised AFP reporting

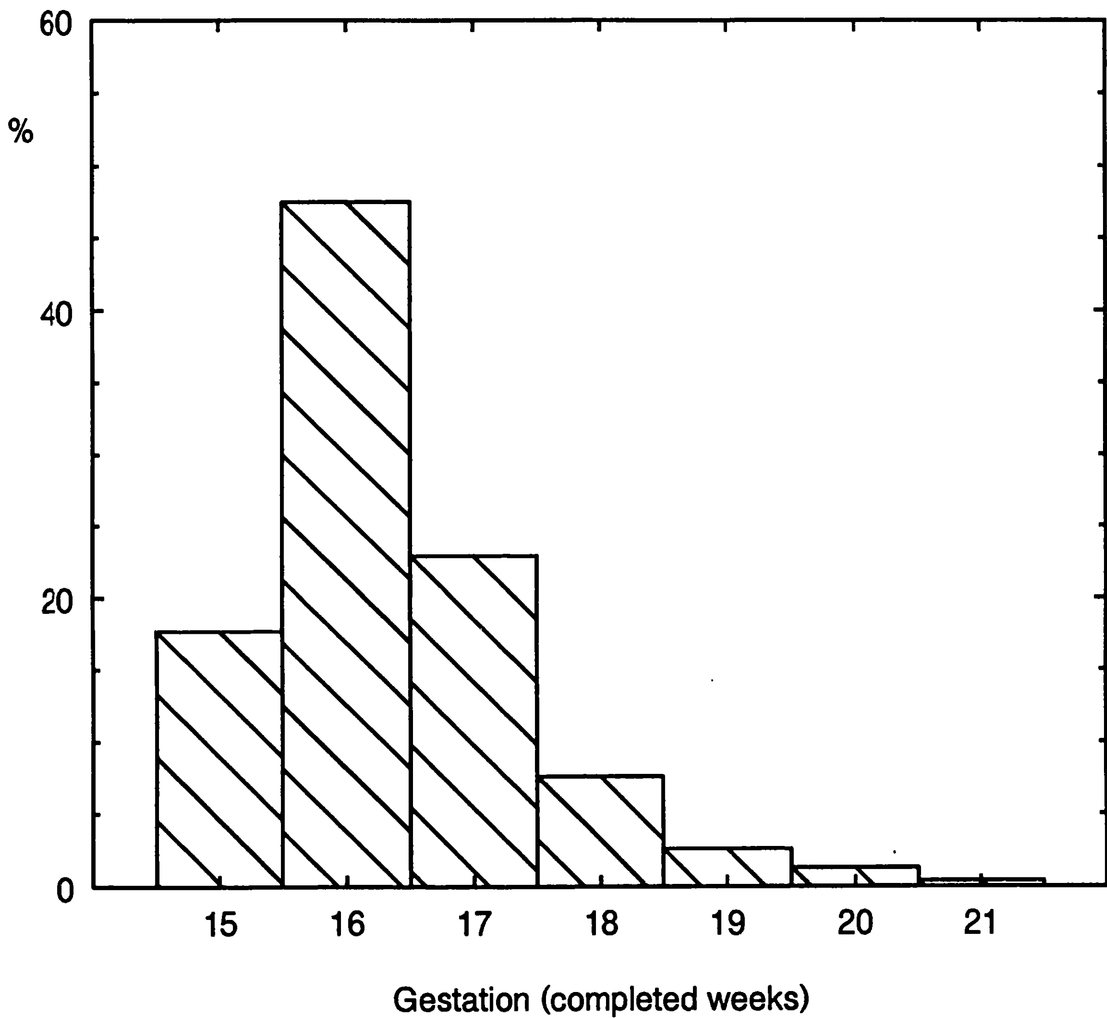
system was required and a new reporting format devised. Consultant obstetricians and antenatal clinic staff were informed of the impending developments by letter and revised patient information leaflets and a guide to interpretation of screening results produced.

Maternal serum AFP and hCG results were measured as described in Sections 4.1.2.2 and 4.1.3.1. Risks for Down's syndrome were calculated using the hCG(MOM)/AFP(MOM) ratio and mid trimester maternal age risks of Down's syndrome, and a threshold risk of 1:220 was selected. The gestational range for screening was extended to 15-21 weeks inclusive.

During the first year of operation 30,084 women were screened and there were approximately 37,500 birth relating to this period. The distribution of gestations at which samples were taken is shown in Figure 5-30, with 88% of samples taken between 15 and 17 completed weeks. The distribution of maternal ages is shown in Figure 5-31. The median age was 26.4 years and 6.9% of women were aged 35 years and over. From cytogenetic records a total of 50 Down's syndrome pregnancies were identified, of which 37 were in the screened population. There were also 10 cases of trisomy 18, of which 7 were in the screened population.

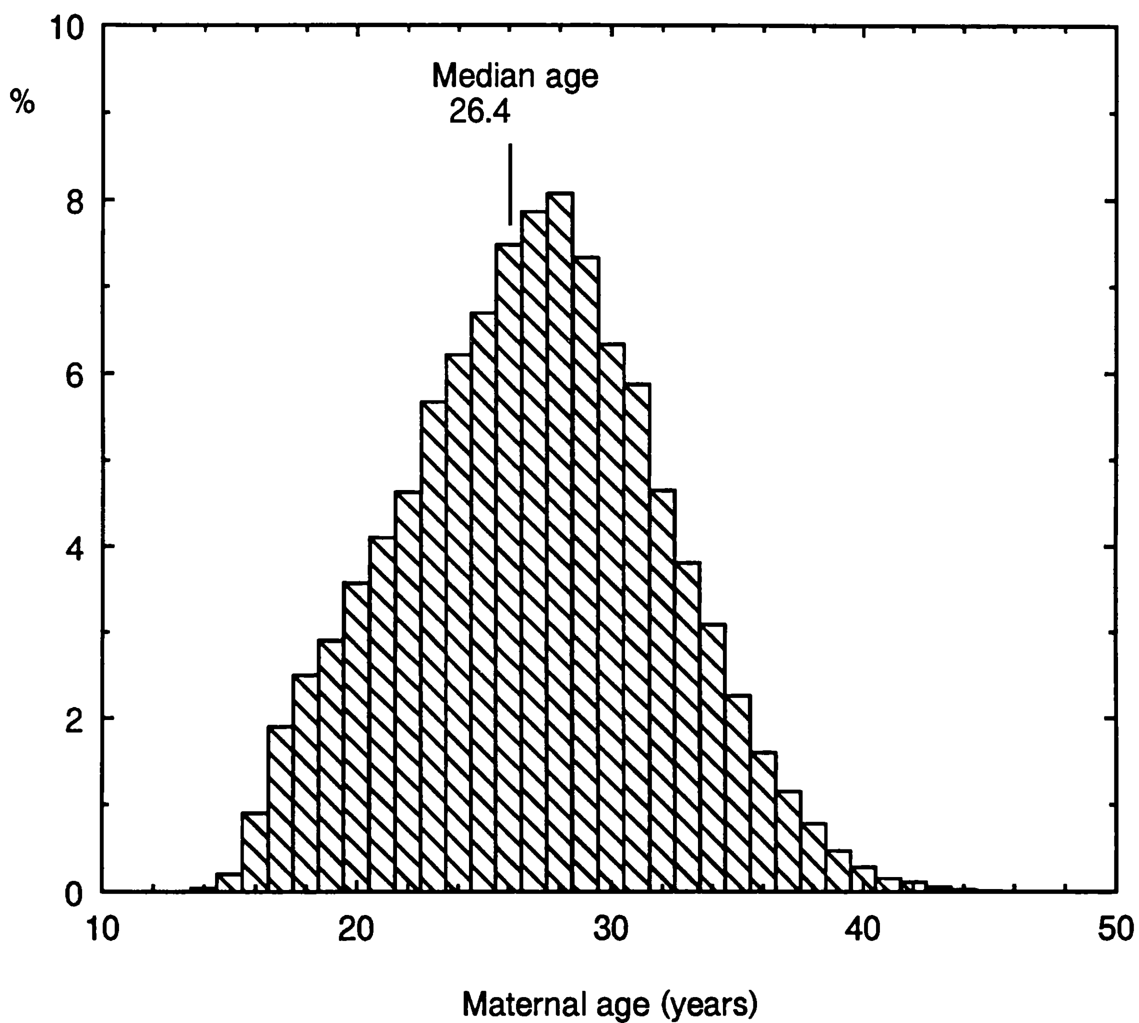
5.3.2.1 SCREENING FOR DOWN'S SYNDROME

A summary of the screening performance is given in Table 5-43. The initial 6.3% false positive rate was reduced to 5.1% after some



<u>Figure 5 - 30</u>

The distribution of gestations at which samples were taken in the 1st year of hCG/AFP/age screening, September 1991 to September 1992.



<u>Figure 5 - 31</u>

The distribution of maternal ages of the women screened in the 1st year of hCG/AFP/age screening, September 1991 to September 1992.

Table 5 - 43

Performance of hCG/AFP/age screening for Down's syndrome in the west of Scotland September 1991 to September 1992.

Total pregnancies screened	: 30,084
*No (%) in high risk group - initial	: 1,904 (6.3%)
-after gestation reassessed	: 1,523 (5.1%)
Uptake of diagnostic testing	: 70%
No of Down's syndrome in screened population	: 37
No (%) in high risk group	: 26 (70%)
(95% CI)	(53-84%)
No (%) prenatally diagnosed	: 21 (56%)
(95% CI)	(39-72%)
Overall odds of being affected if in the high risk group	: 1:59
Overall odds of being affected if in the low risk group	: 1:2600

* (Cut-off risk 1:220)

gestations had been reassessed. The uptake of diagnostic testing was 70%. Of the Down's syndrome pregnancies 26 out of 37 (70%) were in the high risk group and 21 of these (56%) were prenatally diagnosed. The overall odds of an affected pregnancy if in the high risk group were 1:59.

The individual risks for each of the 37 Down's syndrome pregnancies are shown in Table 5-44, classified into high and low risk, and, if in the high risk group, divided into those women who had prenatal diagnosis and those who did not. It can be seen that neither the actual risk nor maternal age were the only factors which influenced whether prenatal diagnosis occurred. These same risks are shown graphically in Figure 5-32, compared with the population mid trimester risk of Down's syndrome and the cut-off risk of 1:220. The detection rate varies with different maternal age groups, as is shown in Table 5-45, from 57% in women aged less than 25 years to 91% in women aged 35 years and over.

A summary of all of the Down's syndrome pregnancies identified cytogenetically from the whole pregnant population relating to this year of screening with hCG/AFP/age is shown in Table 5-46, divided into different maternal age groups. It can be seen that, of the 50 Down's syndrome pregnancies, 24 (48%) were prenatally diagnosed, the majority of these because of an indication of a risk of ≥1:220 from hCG/AFP/age screening and the rest for an indication of maternal age of 35 years or greater. In women aged 35 years or over 11 out of 16 of the Down's syndrome pregnancies (69%) were prenatally diagnosed, and in women aged less than 35 years 13 out of 34 (38%) were prenatally diagnosed.

Table 5 - 44

Risks from hCG/AFP/age for Down's syndrome pregnancies screened September 1991 to September 1992.

High risk group				Low risk group	
Prenatally diagnosed		Born		Born	
Age	Risk	Age	Risk	Age	Risk
20	1:110	23	1:190	21	1: 270
21	1:210	33	1:120	21	1: 950
23	1:110	34	1: 43	23	1:3100
26	1:160	38	1: 17	25	1: 970
30	>1: 43	38	1:160	25	1:1100
30	>1: 43			27	1: 250
30	1: 68[a]			29	1: 500
31	1:100[b]			29	1: 500
31	1:170			30	1:3800
32	1: 50			31	1: 660[c]
34	>1: 22			37	1: 650
34	>1: 22				
34	1: 24				
36	>1: 15				
36	1: 47				
37	1:100				
37	1:140				
38	>1: 10				
38	1: 60				
39	1: 52				
40	1: 44				

[a] Translocation Down's 46XY,-14,+t(14;21)

[b] Mosaic 46XY/47XY+21

[c] Mosaic 46XY/47XY+21

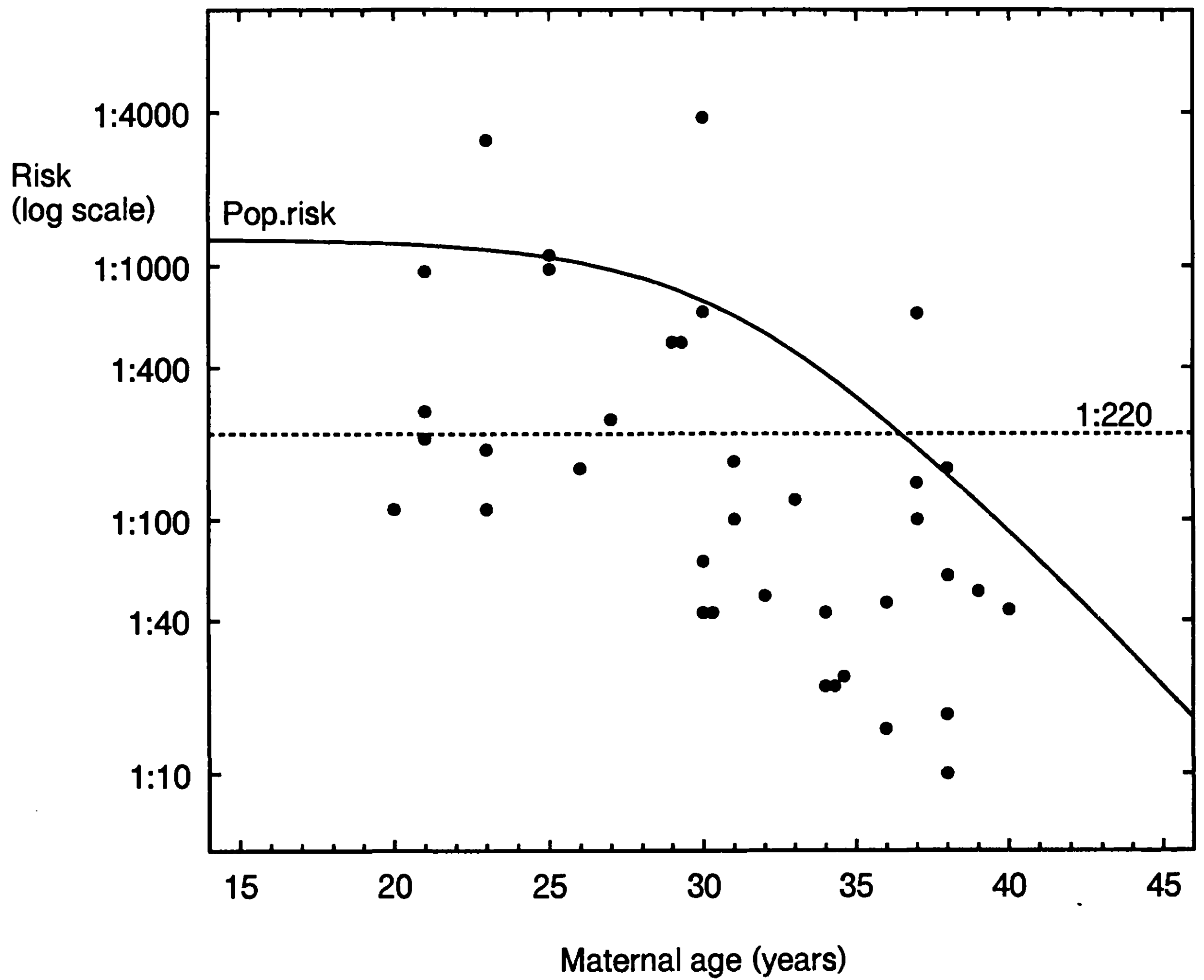


Figure 5 - 32

Combined risks of Down's syndrome at mid trimester, from hCG/AFP/age, in 37 Down's syndrome pregnancies screened between September 1991 and September 1992, compared with the *a priori* maternal age risk and the threshold risk of 1:200.

Table 5 - 45

Efficacy of screening for Down's syndrome using hCG/AFP/age in different maternal age groups.

	Maternal age (years)		
	<25	25-34	≥35
Total Down's	7	19	11
No in high risk group	4	12	10
% in high risk group	57%	63%	91%

Table 5 - 46

Summary of all Down's syndrome pregnancies in the west of Scotland for the period screened September 1991 to September 1992.

	Maternal age			
	<25	25-34	≥35	All
Screened-HR-PND	3	10	8	21
Screened-HR-Born	1	2	2	5
Screened-LR-Born	3	7	1	11
1st Tri CVS-MA-PND	-	-	3	3
Not screened-Born	1	5	2	7
Screened elsewhere -Born	1	1	-	2
Total	9	25	16	50
% in age group	18%	50%	32%	
(Expected %)	(23%)	(46%)	(31%)	

HR = High risk group (Risk ≥1:220)
LR = Low risk group (Risk <1:220)
PND = Prenatally diagnosed
CVS = Chorionic villus sampling
MA = Maternal age ≥35 years

As a check on the completeness of ascertainment of Down's syndrome the expected number of Down's syndrome pregnancies at birth was calculated from the age distribution shown in Figure 5-30. From this 45 Down's syndrome births would be expected (1.2/1000, 95% CI 33-60). However, since there is a defined spontaneous loss of affected pregnancies between the first and second trimester and between the second trimester and birth (see Section 1.2) the number of Down's syndrome births ascertained will be influenced by the stage of pregnancy at which they are diagnosed. Of the 50 Down's syndrome pregnancies identified here, 3 were diagnosed in the 1st trimester, 21 in the 2nd trimester and 26 at birth. Allowing for expected fetal loss this is equivalent to 45 Down's syndrome cases at term, identical to the expected number.

5.3.2.2 SCREENING FOR TRISOMY 18

The very low hCG results in trisomy 18 pregnancies provide an opportunity to define a second high risk group to screen for affected pregnancies. Using the results from 12 trisomy 18 pregnancies, as described in Section 5.3.1, a sliding scale of cut-off values in MOM for hCG different maternal ages was calculated to give a risk of trisomy 18 which was approximately 1:100 or greater (Table 5-47). Using this protocol, 87 pregnancies (0.3%) were assigned to the high risk group for trisomy 18. There were 2 trisomy 18 pregnancies identified in this high risk group from a total of 7 in the screened population, giving a detection rate of 29%. Neither of these was prenatally diagnosed. There were

Table 5 - 47

Cut-off hCG (MOM) for high risk group for trisomy 18 at different maternal ages. If the result is less than or equal to the given cut-off then the patient is assigned to the high risk group.

Maternal age	Cut-off hCG(MOM)
15	0.20
16	0.20
17	0.20
18	0.20
19	0.20
20	0.20
21	0.20
22	0.20
23	0.20
24	0.20
25	0.20
26	0.21
27	0.21
28	0.21
29	0.21
30	0.22
31	0.22
32	0.23
33	0.24
34	0.25
35	0.26
36	0.27
37	0.29
38	0.30
39	0.32
40	0.34
41	0.36
42	0.38
43	0.40
44	0.43
45	0.46

a total of 10 trisomy 18 pregnancies identified cytogenetically for this period and none were prenatally diagnosed. One of the trisomy 18 pregnancies was identified after an intrauterine death at 16 weeks gestation and the remaining 9 were identified at birth.

SECTION 6

DISCUSSION

6.1 ANALYTE LEVELS IN MATERNAL SERUM IN DOWN'S SYNDROME PREGNANCIES

One of the most important factors affecting the usefulness of a particular maternal serum marker as a predictor of Down's syndrome is the shift in median value between the unaffected and Down's syndrome populations. In addition, the spread of the distributions, as defined by the standard deviation, and the level of correlation between markers used together will also affect the discrimination between unaffected and Down's syndrome pregnancies. None of the markers so far studied here or elsewhere show complete separation of the unaffected and Down's syndrome populations.

6.1.1 ALPHAFETOPROTEIN

In the present study a median AFP level of 0.72 MOM was found in 114 Down's syndrome pregnancies. This is the single largest series of Down's syndrome pregnancies investigated for AFP levels. The median maternal serum AFP levels in Down's syndrome pregnancies in 28 studies, including this one, are presented in Table 6-1, along with, where stated, the mean and standard deviation of the AFP log Gaussian distribution. Eliminating three reports where samples were also included in another study, a meta-analysis of 25 studies, comprising 1062 cases of affected pregnancies, gave an overall geometric median of 0.75 MOM, with a range from 0.63 to 1.00 MOM. The median AFP value of 0.72 MOM found in this study is close to the overall geometric median. Only one report, from Cowchock and Ruch (1984) found no difference between levels in

Table 6 -1

Summary of maternal serum alphafetoprotein (AFP) medians, means and standard deviations (SD) found in Down's syndrome pregnancies in published studies.

	n	Median AFP (MOM)	Mean (log_{10})	SD (log_{10})
Merkatz *et al.* (1984)	25	0.68[a]	-	-
Cuckle *et al.* (1984)	61	0.72[b]	-	-
Seller (1984)	8	0.80	-	-
Guibaud *et al.* (1984)	13	0.76	-	-
Tabor *et al.* (1984)	25	0.75[c]	-	-
Fuhrmann *et al.* (1984)	43	0.80	-	-
Voigtlander & Vogel (1984)	29	0.78	-	-
Cowchock & Ruch (1984)	40	1.00	-	-
Hershey *et al.* (1985)	28	0.87	-	-
Spencer & Carpenter (1985)	27	0.82	-	-
Murday & Slack (1985)	45	0.63	-	-
Doran *et al.* (1986)	46	0.79	-	-
Ashwood *et al.* (1987)	26	0.69	-0.212	0.283
Cuckle *et al.* (1987)	68	0.72	-0.1427	0.2052
Tabor *et al.* (1987)	86	0.64	-0.197	0.186
Di Maio *et al.* (1987)	18	0.76	-	-
Del Junco *et al.* (1989)	22	0.64	-	-
Osathanondh *et al.* (1989)	26	0.84	-	-
Heyl *et al.* (1990)	18	0.82	-0.112	0.162
Norgaard Pedersen *et al.*(1990)	42	0.70	-0.154	0.198
Suchy & Yeager (1990)	16	0.70	-	-
Waller *et al.* (1990)	113	0.77	-	-
MacDonald *et al.* (1991)	54	0.90	-	-
Miller *et al.* (1991)	8	0.75	-	-
Spencer (1991)	29	0.73[d]	-0.130	0.208
Ryall *et al.* (1992)	57	0.74	-0.295[e]	0.464[e]
Spencer *et al.* (1992b)	90	0.70	-0.1413	0.2013
This study (Zeitune *et al.*,1991)	113	0.72	-0.1427	0.1626
Overall weighted geometric median	1062	0.75		

[a] Estimated from figure in publication.
[b] Not included in overall analysis as cases also included in Cuckle *et al.* (1987).
[c] Not included in overall analysis as cases also included in Tabor *et al.* (1987).
[d] Not included in overall analysis as cases also included in Spencer *et al.* (1992b).
[e] Log_e - equivalent to log_{10} Mean = -0.1281, SD = 0.2015.

Down's syndrome and unaffected pregnancies, and the consensus is that AFP levels in Down's syndrome cases are lowered to around 75% of that found in unaffected pregnancies.

6.1.2 HUMAN CHORIONIC GONADOTROPHIN

In the present study a median intact hCG level of 2.18 MOM was found in 49 Down's syndrome pregnancies. The maternal serum median hCG levels in Down's syndrome pregnancies found in 18 studies including this one are presented in Table 6-2, along with, where stated, the mean and standard deviation of the log Gaussian distribution. Eliminating two reports where samples were also included in another study a meta-analysis of 16 studies, comprising 634 cases of affected pregnancies, gave an overall geometric median of 2.08 MOM, with a range of 1.57 to 2.91 MOM. The median value of the present study is of 2.18 MOM is close to the overall geometric median. The consensus is that intact hCG levels in Down's syndrome pregnancies are around double those found in unaffected pregnancies, making hCG a powerful predictor of Down's syndrome.

6.1.3 PREGNANCY-SPECIFIC β-1 GLYCOPROTEIN

In the present study a median SP_1 level of 1.17 MOM was found in 48 Down's syndrome pregnancies. The median maternal serum SP_1 level in Down's syndrome pregnancies in five studies, including this one, are shown in Table 6-3. Meta-analysis of these,

Table 6 - 2

Summary of maternal serum chorionic gonadotrophin (hCG) medians, means and standard deviations (SD) found in Down's syndrome pregnancies in published studies.

	n	Median hCG (MOM)	Mean ($\log_{10}$)	SD ($\log_{10}$)
Bogart *et al.* (1987)	29	1.61[a]	-	-
Arab *et al.* (1988)	77	1.61[b]	-	-
Wald *et al.* (1988b)	77	2.04	0.3096	0.2588
Del Junco *et al.* (1989)	22	2.10	-	-
Osathanondh *et al.* (1989)	26	2.59	-	-
Petrocik *et al.* (1989)	38	2.50	-	-
White *et al.* (1989)	15	2.91[b]	-	-
Bartels *et al.* (1990)	43	2.18	-	-
Heyl *et al.* (1990)	18	1.89	0.290	0.283
Muller & Boué (1990)	50	2.39	-	-
Norgaard Pedersen *et al.*(1990)	42	1.57	0.195	0.317
Suchy & Yeager (1990)	16	2.40	-	-
MacDonald *et al.* (1991)	54	1.79	0.25	0.26
Miller *et al.* (1991)	8	2.11	-	-
Spencer (1991)	29	1.88[c]	0.2881	0.2257
Ryall *et al.* (1992)	57	2.12	0.751[d]	0.561[d]
Spencer *et al.* (1992b)	90	2.03	0.3282	0.2825
This study (Crossley *et al.*,1991a)	49	2.18	0.3385	0.3127
Overall weighted geometric median	634	2.08		

[a] Not included in overall analysis as cases also included in Bartels *et al.* (1990).

[b] Mean not median.

[c] Not included in overall analysis as cases also included in Spencer *et al.* (1992b).

[d] $\log_e$ - equivalent to $\log_{10}$ Mean = 0.326, sd = 0.244.

Table 6 - 3

Summary of maternal serum pregnancy-specific ß-1 glycoprotein (SP_1) medians, means and standard deviations (SD) found in Down's syndrome pregnancies in published studies.

	n	Median SP_1 (MOM)	Mean ($\log_{10}$)	SD ($\log_{10}$)
Bartels & Lindemann (1988)	24	2.10	-	-
Wald *et al.* (1989)	77	1.20	-	-
Knight *et al.* (1989)	24	1.53	-	-
Petrocik *et al.* (1990)	46	1.98[a]	-	-
Bartels *et al.* (1990)	43	1.54	-	-
This study (Graham *et al.*,1992)	48	1.17	0.068	0.176
Overall weighted geometric median	262	1.46		

[a] Estimated from figure in publication.

comprising 262 cases of affected pregnancies, gave an overall geometric median of 1.46 MOM, with a range of 1.17 to 2.10 MOM. The median SP_1 level of 1.17 MOM found in this study is the lowest in the series, but is in close agreement with that found by Wald *et al.* (1989), who found a median SP_1 level of 1.20 MOM in a series of 77 down's syndrome pregnancies.

The initial study of Bartels and Lindemann (1988), based on the analysis of post-amniocentesis maternal serum samples from 24 Down's syndrome pregnancies suggested a much stronger association between elevated SP_1 levels and Down's syndrome, with a median value of 2.1 MOM. However an overall median value for unaffected pregnancies was used, rather than individual medians for different weeks of gestation. Subsequent studies (Wald *et al.*, 1989, Petrocik *et al.*, 1990, Bartels *et al.* 1990, present study) have shown that SP_1 levels increase with advancing gestation between 15-20 weeks. Thus, significant error may have arisen through the use of control samples of earlier gestation than those of the Down's syndrome cases.

Some discrepancy is apparent in the SP_1 levels found in the Down's syndrome pregnancies between the various studies. Two studies (Bartels & Lindemann, 1988, Petrocik *et al.*, 1990) found markedly elevated levels, with a median value at around 2.0 MOM. Two others (Knight *et al.*, 1989, Bartels *et al.*, 1990) found moderately elevated levels, with a median SP_1 value at around 1.5 MOM, and the remaining two (Wald *et al.*, 1989, present study) found only a small shift in SP_1 levels in Down's syndrome pregnancies, with a median value at around 1.2 MOM. These differences are not assay

dependent, as four studies (Bartels *et al.*, 1988, Wald *et al.*, 1989, Petrocik *et al.*, 1990, Bartels *et al.*, 1990) all used the same commercially available assay method (Enzygnost-SP1, Behring). The present study and that by Wald *et al.* (1989) have the most representative series of Down's syndrome samples and controls as they were identified from maternal serum population screening programmes, thus avoiding the selection bias found other studies where samples were obtained from pregnancies of advanced maternal age or after prenatal diagnosis of Down's syndrome by amniocentesis. Also, this study and that of Wald *et al.* (1989) have used much larger numbers of control pregnancies (390 and 385 respectively) to assess the normal pregnancy levels of SP_1, compared with the other four reports which had between 34 and 117 control samples.

6.1.4 UNCONJUGATED ESTRIOL

In the present study a median UE3 level of 0.79 MOM was found in 49 Down's syndrome pregnancies. The median maternal serum UE3 levels in Down's syndrome pregnancies in 12 studies including this one are shown in Table 6-4, along with, where stated, the mean and standard deviation of the UE3 distribution, either Gaussian or log Gaussian. Eliminating one report where samples were also included in another study, a meta-analysis of 11 studies, comprising 498 affected pregnancies, gave a overall geometric median of 0.75 MOM, with a range of 0.66 to 0.99 MOM. The median UE3 level of 0.79 MOM found in this study is close to the overall geometric median. Only one report, Macri *et al.* (1990a), found no difference between

Table 6 - 4

Summary of maternal serum unconjugated estriol (UE3) medians, means and standard deviations (SD) found in Down's syndrome pregnancies in published studies.

	n	Median UE3 (MOM)	Mean	SD
Canick *et al.* (1988)	22	0.79	-	-
Wald *et al.* (1988a)	77	0.73	0.73[a]	0.26[a]
Del Junco *et al.* (1989)	22	0.70	-	-
Osathanondh *et al.* (1989)	26	0.66	-	-
Heyl *et al.* (1990)	18	0.77	0.79[a]	0.33[a]
Macri *et al.* (1990a)	41	0.99	-	-
Norgaard pedersen *et al.*(1990)	42	0.74	-0.129[b]	0.202[b]
MacDonald *et al.* (1991)	54	0.71	0.77[a]	0.29[a]
Spencer (1991)	29	0.73[d]	-0.1879[b]	0.2110[b]
Ryall *et al.* (1992)	57	0.70	-0.353[c]	0.411[c]
Spencer *et al.* (1992b)	90	0.74	-0.1601[b]	0.2101[b]
This study (Crossley *et al.*, 1993)	49	0.79	0.79[a]	0.29[a]
Overall weighted geometric median	498	0.75		

[a] Gaussian distribution used

[b] Log Gaussian distribution used

[c] Log_e - equivalent to log_{10} Mean = -0.153, SD = 0.178

[d] Not included in overall analysis as cases also included in Spencer *et al.* (1992b)

Down's syndrome and unaffected pregnancies, and the consensus is that UE3 levels are lowered to around 75% of those found in unaffected pregnancies, similar to AFP.

Some controversy exists as to whether the distribution of maternal serum UE3 in Down's syndrome and unaffected pregnancies fits a Gaussian or log Gaussian distribution. Four reports (Wald *et al.*, 1988a, Heyl *et al.*, 1989, MacDonald *et al.*, 1991, present study) fit their data to a Gaussian distribution, while 3 (Norgaard Pederson *et al.*, 1990, Ryall *et al.*, 1992, Spencer *et al.*, 1992b) use a log Gaussian distribution. One group of authors (Wald *et al.*, 1988a) have subsequently revised their data to fit a log Gaussian distribution rather than Gaussian distribution (Wald *et al.*, 1992). The present study has shown that the distribution of UE3 in Down's syndrome and unaffected pregnancies does not ideally fit either a Gaussian or Log Gaussian distribution (Section 5.2.1.3.2).

6.1.5 FREE ß HUMAN CHORIONIC GONADOTROPHIN

In the present study a median Frß-hCG level of 2.30 MOM was found in 81 Down's syndrome pregnancies. The median maternal serum Frß-hCG levels in Down's syndrome pregnancies in seven studies, including this one, are presented in Table 6-5, along with, where stated, the mean and standard deviation of the Frß-hCG log Gaussian distribution. Eliminating two reports where samples were also included in another study, a meta-analysis of 5 studies, consisting of 332 cases of affected pregnancies, gave an overall

Table 6 - 5

Summary of maternal serum free ß human chorionic gonadotrophin (Frß-hCG) medians, means and standard deviations (SD) found in Down's syndrome pregnancies in published studies.

	n	Median Frß-hCG (MOM)	Mean (log_{10})	SD (log_{10})
Macri *et al.* (1990b)	29	2.31[a]	-	-
Spencer (1991)	29	2.06[b]	0.3061	0.2744
Spencer & Macri (1992)	23	2.15[b]	-	-
Ryall *et al.* (1992)	57	2.36	0.860[c]	0.693[c]
Spencer *et al.* (1992b)	90	2.41	0.3570	0.3316
Wald *et al.* (1993a)	75	2.22	0.3513	0.3461
This study (Crossley *et al.*, 1991b)	81	2.30	0.362	0.355
Overall weighted geometric median	332	2.32		

[a] Estimated from figure in publication.

[b] Not included in overall analysis as cases also included in Spencer *et al.* (1992b).

[c] Log_e - equivalent to log_{10} Mean = 0.373, SD = 0.301

geometric median of 2.32 MOM, with a range of 2.22 to 2.41 MOM. The median Frβ-hCG value of 2.30 found in the present study is close to the overall geometric median. The consensus is that Frβ-hCG levels in Down's syndrome pregnancies exceed twice the level found in unaffected pregnancies, making Frβ-hCG a powerful predictor of Down's syndrome.

6.1.6 OTHER ANALYTES

Free α subunit hCG. There have been five reported studies of free α subunit hCG (Frα-hCG) levels in Down's syndrome pregnancies, which are summarised in Table 6-6. Three studies (Bogart *et al.*, 1987, Bogart *et al.*, 1989, and Kratzer *et al.*, 1991), totalling 33 cases, found median levels of Frα-hCG >2.0 MOM in Down's syndrome pregnancies. The study by Ryall et al. (1992), with 57 cases of Down's syndrome, found moderately elevated levels of Frα-hCG in Down's syndrome with a median level of 1.39 MOM. The study by Spencer (1993), with 36 cases of Down's syndrome, found no difference in levels between Down's syndrome cases and controls. There is also considerable variation in the spread of values found, with Bogart *et al.* (1987) and Bogart *et al.* (1989) reporting ranges of 1.25 - 3.69 MOM and 0.56 - 2.96 MOM respectively. Ryall *et al.* (1992) reported a ±2 SD range of 0.69 - 2.81 MOM, compared with the considerably smaller ±2 SD range of 0.93 - 1.04 MOM reported by Spencer (1993). Some doubt must therefore exist as to whether the assays used in these different studies had the same specificity, and further work is required to assess the extent of Frα-hCG variation in Down's syndrome

Table 6 - 6

Summary of maternal serum free α human chorionic gonadotrophin (Frα-hCG) medians, means and standard deviations (SD) found in Down's syndrome pregnancies in published studies

	n	Median Frα-hCG (MOM)	Mean $\log_{10}$	SD $\log_{10}$
Bogart *et al.* (1987)	17	2.31		
Bogart *et al.* (1989)	13	1.82		
Kratzer *et al.* (1991)	3	2.02		
Ryall *et al.* (1992)	57	1.39	0.330[a]	0.351[a]
Spencer (1993)	36	0.991	0.989	0.028

[a] $\log_e$ - equivalent to $\log_{10}$ Mean = 0.143, SD = 0.152

pregnancies.

Progesterone. There have been three reports on maternal serum progesterone levels in Down's syndrome pregnancies. Moderately elevated levels have been reported by Knight *et al.* (1989) (1.34 MOM in 24 cases) and Kratzer *et al.* (1991) (1.49 MOM in 3 cases). Ryall *et al.* (1992) analysed progesterone in 57 cases of Down's syndrome. The median level of progesterone found was not reported but the difference between affected and unaffected cases was not significant by t-test ($p>0.05$). Knight *et al* (1989) reported high levels of correlation between progesterone and the other placental markers, hCG, SP_1 and human placental lactogen (hPL).

Human Placental Lactogen. There have been two studies of maternal serum hPL levels in Down's syndrome pregnancies. Knight *et al.* (1989), found elevated levels in 24 cases, with a median value of 1.79 MOM, whereas Ryall *et al.* (1992) found moderately elevated levels in 57 cases, with a median value of 1.19 MOM.

Pregnancy Associated Plasma Protein A. Pregnancy associated plasma protein A (PAPP-A) levels in Down's syndrome in the second trimester of pregnancies appear to be virtually unchanged from the levels found in unaffected pregnancies. Cuckle *et al.* (1992), in 18 Down's syndrome pregnancies, reported a median level of 0.87 MOM. Wald and Voller (1992), in 16 Down's syndrome cases, found a median level of 1.02 MOM, and Knight *et al.* (1993), in 30 cases of Down's syndrome, found a median level of 1.01 MOM. PAPP-A is therefore not a useful predictor of Down's syndrome in the second trimester.

Cancer Antigen 125. Reports on cancer antigen 125 (CA125) in Down's syndrome pregnancies in the second trimester are conflicting. One study (Hogdall *et al.*, 1992), of 15 Down's syndrome pregnancies and 60 controls, reported elevated levels but did not give a median MOM value. Van Blerk *et al.* (1992), reported reduced, but not significantly different, levels of CA125 in a study of 10 Down's syndrome pregnancies and 78 controls.

Inhibin. Inhibin, which is involved in the endocrine control of gonadotrophin secretion (Petraglia *et al.*, 1989), has been reported to be elevated in maternal serum from Down's syndrome pregnancies (Van Lith *et al.*, 1992). In a study of 10 Down's syndrome pregnancies the median inhibin level was 1.9 MOM, a statistically significant difference. Thus inhibin may be a potentially useful marker for Down's syndrome, and further studies are indicated to investigate its association with the other placental markers hCG and Frß-hCG.

Thyroid Auto-antibodies. Thyroid auto-antibodies were observed by Fialkow *et al.* (1965) to be higher in women who had previously had a Down's syndrome pregnancy. Cuckle *et al.* (1988a) followed up this observation and found higher, but not statistically significantly different, levels of thyroid antibodies in maternal serum samples from pregnancies affected by Down's syndrome. The authors attribute the lesser effect on thyroid antibodies in women currently pregnant with a Down's syndrome fetus to the fact that the immune system is depressed during pregnancy (Sridama *et al.*, 1982), and also that thyroid antibody levels decrease on average

in women with established thyroid auto-immune disease who become pregnant (Amino *et al.*, 1978).

Urea resistant neutrophil alkaline phosphatase. Grozdea *et al.* (1984) observed a significant increase in urea resistant neutrophil alkaline phosphatase (URNAP) activity in the mothers of Down's syndrome children. Cuckle *et al.* (1990a) studied samples taken prior to termination of pregnancy, from 72 women who had a Down's syndrome fetus prenatally diagnosed by amniocentesis or CVS. Using an arbitary scoring system, median enzyme activity in the Down's syndrome cases was 1.65 MOM of 156 unaffected pregnancies, 46 of whom had had an invasive diagnostic procedure and 110 of whom had not. Although a potentially promising discriminator of Down's syndrome the measurement of URNAP has several practical problems making it unsuitable for use in routine population screening. The first is the necessity for collecting blood films rather than blood samples for analysis. The second is that this technique is far more labour intensive than other assay methods used in routine screening, such as RIA or IRMA, with 100 cells on each slide having to be scored by microscopic examination for the level of staining in each cell. The third is the subjective nature of the test, which is shown in this study by the observation that two different operators had an average difference in score of 55, while the median scores for unaffected pregnancies between 15 and 20 weeks gestation are in the range 70-85. No information is currently available on the level of correlation between URNAP and other pregnancy markers.

6.1.7 CORRELATION BETWEEN ANALYTES IN UNAFFECTED AND DOWN'S SYNDROME PREGNANCY SAMPLES

Significant association between two markers reduces the predictive value of the markers as indicators of chromosomally abnormal pregnancies when the are used together to derive risks.

The levels of correlation reported in various studies between the analytes AFP, UE3, hCG and Frß-hCG are summarised in Tables 6-7 to 6-12. For the AFP/UE3 combination (Table 6-7) correlation coefficients ranging from 0.13 to 0.33 have been reported in samples from unaffected pregnancies while in Down's syndrome pregnancies correlation coefficients range from 0.08 to 0.478. In five out of seven of these control sample sets, including the present study, and in four out of seven of the Down's syndrome sample sets, including the present study, the levels of correlation are statistically highly significant.

Eight studies have reported on the level of correlation between AFP and hCG (Table 6-8). Six of these gave the correlation coefficient for Down's syndrome sets, ranging from -0.118 to 0.46. Only one of these (Heyl *et al.*, 1990) found significant correlation ($0.01<p<0.05$). Of the eight control sets, correlation coefficients ranged from 0.05 to 0.164, and in two studies (Spencer *et al.*, 1992, Ryall *et al.*, 1992) the level of correlation was highly significant ($p<0.01$). The present study found lower but still significant levels of correlation ($0.01<p<0.05$) in the control samples only.

Table 6 - 7

Summary of correlation coefficients (r) found between AFP and UE3 in Down's syndrome and control pregnancies in published studies.

	Controls		Down's syndrome	
	n	r	n	r
Wald *et al.* (1988a)	385	0.13[a]**	77	0.14[a]
Osathanondh *et al.* (1989)	23	<0.26	26	<0.26
Heyl *et al.* (1990)	85	0.13[a]	18	0.08[a]
Norgaard Pedersen *et al.*(1990)	291	0.33[b]**	42	0.36[b]**
Spencer (1991)	145	0.189[bd]*	29	0.624[bd]**
Spencer *et al.* (1992b)	2862	0.305[b]**	90	0.374[b]**
Ryall *et al.* (1992)	390	0.317[c]**	57	0.478[c]**
This study (Crossley *et al.*, 1993)	390	0.25[a]**	49	0.44[a]**

[a] Correlation coefficient between logAFP-UE3.

[b] Correlation coefficient between logAFP-logUE3.

[c] Correlation coefficient between $\log_e$AFP-$\log_e$UE3.

[d] Samples also included in Spencer *et al.* (1992b).

* Significant correlation ($0.01<p<0.05$).

** Highly significant correlation ($p<0.01$).

Table 6 - 8

Summary of correlation coefficients (r) found between logAFP and loghCG in Down's syndrome and control pregnancies in published studies.

	Controls		Down's syndrome	
	n	r	n	r
Wald *et al.* (1988b)	385	0.05	77	0.14
Osathanondh *et al.* (1989)	23	<0.26	26	<0.26
Petrocik *et al.* (1989)	125	0.05	-	-
Heyl *et al.* (1990)	85	0.16	18	0.46*
Suchy & Yeager (1990)	614	0.033	-	-
Spencer (1991)	145	0.280[a]**	29	-0.119[a]
Spencer *et al.* (1992b)	2862	0.152**	90	-0.118
Ryall *et al.* (1992)	390	0.164[b]**	57	0.049[b]
This study (Crossley *et al.*, 1991a)	410	0.11*	49	0.12

[a] Samples also included in Spencer *et al.* (1992b).

[b] $\log_e$AFP-$\log_e$hCG

* Significant correlation ($0.01 < p < 0.05$).

** Highly significant correlation ($p < 0.01$).

For the AFP/Frβ-hCG combination five studies have reported on the level of correlation (Table 6-9) but none found statistically significant association.

For the UE3/hCG combination (Table 6-10) reported in six studies, correlation coefficients for the samples from unaffected pregnancies ranged from 0.01 to -0.216 and in Down's syndrome pregnancies from 0.11 to -0.3267. The level of correlation for the control samples was highly significant ($p<0.01$) in one study and approaching significance ($0.01<p<0.05$) in two others, including this study. In the Down's syndrome samples two studies show some correlation ($0.01<p<0.05$) and one highly significant correlation ($p<0.01$).

Of the four studies, including this one, reporting on correlation for the UE3/Frβ-hCG combination (Table 6-11) only one reported highly significant correlation ($p<0.01$) in the Down's syndrome pregnancies, whereas three out of four, including the present study, reported highly significant correlation ($p<0.01$) in the control samples. Correlation coefficients ranged from 0.06 to -0.3323 in the Down's syndrome samples and from 0.027 to -0.232 in the control samples.

For the hCG/Frβ-hCG combination (Table 6-12) all four studies, including this one, reported highly significant levels of correlation ($p<0.01$) on both control and Down's syndrome samples, with correlation coefficients ranging from 0.682 to 0.82 in the control samples and from 0.74 to 0.9376 in the Down's syndrome samples.

Table 6 - 9

Summary of correlation coefficients (r) found between logAFP and logFrβ-hCG in Down's syndrome and control pregnancies in published studies.

	Controls		Down's syndrome	
	n	r	n	r
Macri *et al.* (1990)	450	-0.05	29	0.11
Spencer (1991)	145	0.0884[a]	29	0.0205[a]
Spencer *et al.* (1992b)	2862	0.019	90	0.184
Ryall *et al.* (1992)	390	0.040[b]	57	-0.023[b]
Wald *et al.* (1993a)	367	0.0017	75	0.1481
This study (Crossley *et al.*, 1991b)	390	0.07	81	0.08

[a] Samples also included in Spencer *et al.* (1992b).

[b] $\log_e$AFP-$\log_e$hCG

* Significant correlation ($0.01<p<0.05$).

** Highly significant correlation ($p<0.01$)

Table 6 - 10

Summary of correlation coefficients (r) found between UE3 and hCG in Down's syndrome and control pregnancies in published studies.

	Controls		Down's syndrome	
	n	r	n	r
Wald *et al.* (1988b)	385	-0.08[a]	77	-0.25[a]*
Osathanondh *et al.* (1989)	23	<0.26	26	<0.26
Heyl *et al.* (1990)	85	0.01[a]	18	0.46[a]*
Spencer (1991)	145	-0.1616[bd]*	29	-0.3267[bd]
Spencer *et al.* (1992b)	2862	-0.119[b]**	90	-0.293[b]**
Ryall *et al.* (1992)	390	-0.216[c]*	57	-0.220[c]
This study (Crossley *et al.*, 1993)	390	-0.13[a]*	49	0.11[a]

[a] Correlation coefficient between loghCG-UE3.

[b] Correlation coefficient between logHCG-logUE3.

[c] Correlation coefficient between $\log_e$hCG-$\log_e$UE3.

[d] Samples also included in Spencer *et al.* (1992b).

* Significant correlation ($0.01<p<0.05$).

** Highly significant correlation ($p<0.01$).

Table 6 - 11

Summary of correlation coefficients (r) found between UE3 and Frβ-hCG in Down's syndrome and control pregnancies in published studies.

	Controls		Down's syndrome	
	n	r	n	r
Spencer (1991)	145	-0.2132[bd]**	29	-0.0969[bd]
Spencer *et al.* (1992b)	2862	0.027[b]	90	0.052[b]
Ryall *et al.* (1992)	390	-0.232[c]**	57	-0.221[c]
Wald *et al.* (1993a)	367	-0.1327[b]**	75	-0.3323[b]**
This study (Crossley *et al.*, 1991b)	390	-0.14a**	49	0.06[a]

[a] Correlation coefficient between logFrβ-hCG-UE3.

[b] Correlation coefficient between logFrβ-HCG-logUE3.

[c] Correlation coefficient between $\log_e$Frβ-hCG-$\log_e$UE3.

[d] Samples also included in Spencer *et al.* (1992b).

* Significant correlation ($0.01<p<0.05$).

** Highly significant correlation ($p<0.01$).

Table 6 - 12

Summary of correlation coefficients (r) found between loghCG and logFrß-hCG in Down's syndrome and control pregnancies in published studies.

	Controls		Down's syndrome	
	n	r	n	r
Spencer (1991)	145	0.8901[a]**	29	0.8924[a]**
Spencer *et al.* (1992b)	2862	0.817**	90	0.805**
Ryall *et al.* (1992)	390	0.682[b]**	57	0.907[b]**
Wald *et al.* (1993)	367	0.7382**	75	0.9376**
This study (Crossley *et al.*, 1991b)	390	0.82**	49	0.74**

[a] Samples also included in Spencer *et al.* (1992b).

[b] $\log_e$Frß-hCG-$\log_e$hCG

* Significant correlation ($0.01<p<0.05$).

** Highly significant correlation ($p<0.01$)

In summary there appears to be a very high level of correlation between hCG and Frβ-hCG and virtually none between AFP and Frβ-hCG. The majority of reports found significant correlation between AFP and UE3, and significant correlation was reported in some between UE3 and hCG and UE3 and Frβ-hCG, and in a few between AFP and hCG.

Interpretation of correlation coefficients for significant correlation is dependent on sample size. Correlation coefficients which are significant in a large sample set may not be so in a small sample set. The calculated value of the correlation coefficient (r) is affected by the standard deviation of the analyte values and variations in standard deviation between studies may alter the level of correlation found.

6.2 ANALYTE LEVELS IN MATERNAL SERUM FROM TRISOMY 18 PREGNANCIES

The median levels of the analytes AFP, hCG, SP1, UE3 and Frβ-hCG from published studies are shown in Table 6-13. Much less data is available from trisomy 18 pregnancies than from Down's syndrome, mainly due to the much lower incidence of trisomy 18.

The overall weighted geometric median for AFP in trisomy 18 pregnancies was 0.64 MOM from 164 pregnancies from 13 different reports, including this one. The value of 0.68 MOM found in this study was close to the overall value.

Table 6 - 13

Summary of analyte medians found in trisomy 18 pregnancies in published studies.

		Median				
	n	AFP MOM	hCG MOM	SP_1 MOM	UE3 MOM	Frβ-hCG MOM
Merkatz *et al.* (1984)	13	0.65[a]	-	-	-	-
Hershey *et al.* (1985)	3	0.77[a]	-	-	-	-
Doran *et al.* (1986)	10	0.64	-	-	-	-
Lindenbaum *et al.* (1987)	38	0.60[b]	-	-	-	-
Bogart *et al.* (1987)	3	0.73	0.13	-	-	-
Bartels & Lindemann (1988)	4	-	-	1.47[a]	-	-
Bogart *et al.* (1989)	2	-	0.13	-	-	-
Norgaard Pedersen *et al.* (1990)	7	0.49	-	-	0.38	-
Canick *et al.* (1990a)	10	0.57[b]	0.27	-	0.49	-
Bartels *et al.* (1990)	12	-	0.14	1.25[a]	-	-
Darnule *et al.* (1990)	12	0.75[b]	0.30	-	0.65	-
Miller *et al.* (1991)	9	0.49	0.36	-	-	-
Bogart *et al.* (1991)	2	-	0.48	-	-	-
Blitzer *et al.* (1991)	13	0.79	0.27	-	0.41	-
Staples *et al.* (1991)	12	0.68	0.34	-	0.55	0.31

(continued on next page)

Table 6 - 13 (contd)

	n	Median AFP MOM	hCG MOM	SP_1 MOM	UE3 MOM	Frβ-hCG MOM
Barkai *et al.* (1993)	15	0.66	0.23	-	0.40	-
This study (Zeitune *et al.*, 1991)	19	0.68[b]	-	-	-	-
This study (Crossley *et al.*, 1991a)	12	-	0.21	-	-	-
This study (Crossley *et al.*, 1991b)	11	-	-	-	-	0.23
This study (Graham *et al.*, 1992)	9	-	-	0.87	-	-
This study (Crossley *et al.*, 1993)	4	-	-	-	0.38	-
Overall weighted geometric median (n)		0.64 (164)	0.25 (102)	1.13 (25)	0.47 (73)	0.27 (23)

[a] Estimated from figure in publication.

[b] States that excludes open neural tube defects and anterior abdominal wall defects

For hCG the overall weighted geometric median was 0.25 MOM in 102 pregnancies from 11 different reports, including this one. The value of 0.21 MOM found in this study was close to the overall value. For Frß-hCG the overall geometric median was similar , at 0.27 MOM, in 23 cases from two different reports, including this one.

In contrast the other placental marker, SP1, with the same site of synthesis as intact hCG and Frß-hCG, does not seem to show any alteration in value in trisomy 18 pregnancies. From three reports, including the present study, an overall geometric median of 1.13 MOM was found in 25 pregnancies. The three reports had fairly differing median MOM values for SP1 in trisomy 18 pregnancies, with a range of 0.87 to 1.47 MOM. These same authors (Bartels & Lindemann, 1988, Bartels *et al.*, 1990, present study) also reported differing median values in Down's syndrome pregnancies.

Levels of UE3 were found to be lowered in trisomy 18, with an overall geometric median of 0.47 MOM in 73 pregnancies from seven different reports, including this one. The value of 0.43 MOM found in this study is close to the overall median.

Thus intact hCG and Frß-hCG are the most powerful predictors of trisomy 18, followed by UE3 and then AFP.

6.3 PREDICTED DETECTION RATES FOR DOWN'S SYNDROME FROM RETROSPECTIVE STUDIES, USING BIOCHEMICAL MARKERS

Single analytes in combination with maternal age.

Table 6-14 compares predicted detection rates for Down's syndrome using risks derived from one analyte combined with maternal age risks. Using AFP/age the present study predicted a 37% detection rate for Down's syndrome at a corresponding 6.6% false positive rate (Section 5.1.2.4). Predicted detection rates for Down's syndrome, and corresponding false positive rates from other published studies are shown in Table 6-14. Because of the varying false positive rates quoted it is difficult to compare these exactly, but overall the average detection rate is 36% at a corresponding false positive rate of 7.0%.

Considering UE3/age, the present study predicted a detection rate for Down's syndrome of 29% at a 5% false positive rate. This is compared with other published studies in Table 6-14. Again it is difficult to compare these due to the large differences in false positive rate, but overall the average detection rate is 37% at a corresponding false positive rate of 8.0%

The hCG/age combination in the present study gave a better predicted detection rate for Down's syndrome than either AFP/age or UE3/age, with a predicted detection rate for Down's syndrome of 51% at a corresponding 5% false positive rate. This is compared with other published studies in Table 6-14. Again there is a wide range of false positive rates, but the three studies (Wald *et al.*, 1988b, Spencer *et al.*, 1992b, present study) which quoted false

Table 6 - 14

Predicted detection rates for Down's syndrome and corresponding false positive rates in whole pregnant populations, for single analytes in combination with maternal age, from published studies.

	False positive rate	Det^n rate	Calculation method
AFP/age			
Tabor *et al.* (1987)	9.4	53	BT
Cuckle *et al.* (1987)	5.4	36	LR
MacDonald *et al.* (1991)	6.5	32	LR
Ryall *et al.* (1992)	7.2	25	RM
Spencer *et al.* (1992b)	6.6	31	LR
Present study (Zeitune *et al.* ,1991)	6.6	37	LR
UE3/age			
Wald *et al.* (1988a)	5.2	41	LR
MacDonald *et al.* (1992)	17.0	48	LR
Ryall *et al.* (1992)	7.2	31	RM
Spencer *et al.* (1992b)	5.4	38	LR
Present study (Crossley *et al.*, 1993)	5.0	29	LR
hCG/age			
Wald *et al.* (1988b)	5.0	49	LR
MacDonald *et al.* (1991)	8.0	46	LR
Ryall *et al.* (1992)	9.8	35	RM
Spencer *et al.* (1992)	4.9	45	LR
Present study (Crossley *et al.*, 1991)	5.0	51	LR
Frß-hCG/age			
Macri *et al.* (1990b)	5.0	57	DA
Ryall *et al.* (1992)	8.4	41	RM
Spencer *et al.* (1992b)	5.0	54	LR
Wald *et al.* (1993a)	5.0	56	LR
Present study (Crossley *et al.*, 1991b)	5.0	44	LR

Calculation methods: LR = Likelihood ratio RM = Regression model
BT = Bayes theorem DA = Discriminant analysis

positive rates at or close to 5% had similar predicted detection rates for Down's syndrome, with a range of 45-51%.

Considering the Frß-hCG/age combination, the present study gave a predicted detection rate for Down's syndrome of 44%, at a false positive rate of 5%. Table 6-14 compares this with other published studies. Four of the five studies (Macri *et al.*, 1990, Spencer *et al.*, 1992b, Wald *et al.*, 1993a, present study) stated detection rates for Down's syndrome at a 5% false positive rate, and these had a range from 44-57%.

Thus intact hCG and Frß-hCG are better predictors of Down's syndrome than AFP and UE3 when used in combination with maternal age.

Double analytes in combination with maternal age.

Table 6-15 compares predicted detection rates for Down's syndrome, and corresponding false positive rates, using combinations of two analytes, all including AFP, together with maternal age. From the present study hCG gave the highest predicted detection rate for Down's syndrome, when used in combination with AFP and age, and UE3 the lowest. The predicted detection rates, at a 5% false positive rate are 57% for AFP/hCG/age, 51% for AFP/Frß-hCG/age and 33% for AFP/UE3/age. Other studies similarly found lower detection rates for Down's syndrome using UE3 instead of either intact or free ß hCG (Wald *et al.*, 1988b, 1993, Norgaard-Pedersen *et al.*, 1990, Spencer *et al.*, 1992b). Spencer *et al.* (1992b) and Wald *et al.* (1993), who both compared the predicted performance of intact versus free ß hCG, found improved performance in the predicted

Table 6 - 15

Predicted detection rates for Down's syndrome and corresponding false positive rates in whole pregnant populations, for pairs of analytes in combination with maternal age, from published studies.

	False positive rate	Det^{n} rate	Calculation method
AFP/hCG/age			
Wald *et al.* (1988b)	5.0	55	LR
Norgaard-Pedersen *et al.*(1991)	7.8	55	DA
MacDonald *et al.* (1991)	6.2	48	LR
Ryall *et al.* (1992)	6.4	38	RM
Spencer *et al.* (1992b)	4.9	51	LR
Present study (Crossley *et al.*, 1991a)	5.0	57	LR
AFP/UE3/age			
Wald *et al.* (1988a)	5.2	45	LR
MacDonald et al. (1991)	14.1	43	LR
Ryall *et al.* (1992)	8.9	47	RM
Spencer et al. (1992b)	5.6	35	LR
Present study (Crossley *et al.*, 1993)	5.0	33	LR
AFP/Frβ-hCG/age			
Macri *et al.* (1990)	5.0	72	LR
Spencer *et al.* (1992b)	5.1	60	LR
Wald *et al.* (1993a)	5.0	58	LR
Present study (Crossley *et al.*, 1991b)	5.0	51	LR

Calculation methods: LR = Likelihood ratio
RM = Regression model
DA = Discriminant analysis

detection rate for Down's syndrome using Frß-hCG. This is in contrast to the findings of the present study. The poorer performance of Frß-hCG in the present study may be attributed to the poor precision of the assay used in this study (Section 4.1.4.2), which increased the spread of values and hence the standard deviation of the Frß-hCG values in unaffected and Down's syndrome pregnancies (Table 5-22).

Triple analytes in combination with maternal age.

Table 6-16 summarises published studies which gave predicted detection rates and corresponding false positive rates for combinations of three analytes, either AFP/UE3/hCG/age or AFP/UE3/Frß-hCG/age. The present study found reduced predicted detection rates for Down's syndrome, at a 5% false positive rate, when UE3 was added to either AFP/hCG/age or AFP/Frß-hCG/age. A detection rate of 53% was predicted with AFP/UE3/hCG/age and 57% with AFP/hCG/age. For the AFP/UE3/Frß-hCG/age combination the predicted detection rate was 49% compared with 51% with AFP/Frß-hCG/age. Spencer *et al.* (1992b) noted a similar effect with and without the use of UE3. A detection rate of 51% obtained using AFP/hCG/age could be matched when UE3 was added only at the expense of increasing the false positive rate from 4.9% to 5.3%. Other studies (Wald *et al.*, 1988b, Norgaard-Pedersen *et al.*, 1990) have found an improvement in the predicted detection rate for Down's syndrome when UE3 is used in addition to AFP/hCG/age. The improved detection rates reported by others (MacDonald *et al.*, 1991, Ryall *et al.*, 1992) by the addition of UE3 have been paralleled by an increase in the false positive rate. Of the two studies which compared the addition of UE3 to the AFP/Frß-hCG/age

Table 6 - 16

Predicted detection rates for Down's syndrome and corresponding false positive rates in whole pregnant populations, for combinations of three analytes in combination with maternal age, from published studies.

	False positive rate	Det^{n} rate	Calculation method
AFP/UE3/hCG/age			
Wald *et al.* (1988b)	5.0	61	LR
Norgaard-Pedersen *et al.*(1990)	7.3	58	DA
Macdonald *et al.* (1991)	7.7	60	LR
Ryall *et al.* (1992)	7.9	43	RM
Spencer *et al.* (1992b)	5.3	51	LR
This study (Crossley *et al.*, 1993)	5.0	53	LR
AFP/UE3/Frß-hCG/age			
Spencer *et al.* (1992b)	5.1	60	LR
Wald *et al.* (1993a)	5.0	62	LR
This study	5.0	49	LR

Calculation methods: LR = Likelihood ratio
RM = Regression model
DA = Discriminant analysis

combination, Wald *et al.* (1993a) found a small improvement in the predicted detection rate for Down's syndrome with the addition of UE3, but Spencer *et al.* (1992b) found no improvement.

6.3.1 IS UE3 USEFUL IN SCREENING FOR DOWN'S SYNDROME?

This present study fails to show any improvement in the predicted detection rate for Down's syndrome by the addition of UE3 to that obtainable either by the AFP/hCG/age combination or the AFP/Frß-hCG/age combination. This is attributable to the high level of correlation found between UE3 and AFP which causes a decrease in the likelihood ratios derived from the Down's syndrome distribution in this series (Table 6-17). The contribution to overall risk estimates by additional markers is dependent on several parameters: the shift in the mean value of the affected pregnancies, the spread of the distributions of values in affected and unaffected pregnancies and on the level of correlation between any analytes used together. Significant associations between markers, as found for UE3 and AFP, will reduce their predictive value when used in combination.

The study by Wald *et al.* (1988a) showed a more modest association between AFP and UE3 than that reported here (0.13 vs 0.25 in the controls and 0.14 vs 0.44 in the Down's pregnancies). These authors used a modified Amersham AMERLEX RIA (designated IM2) originally optimised for use in the third trimester. Levels of correlation similar to those found by Wald *et al.* (1988a) have been reported by Heyl *et al.* (1990) who also used the modified IM2

Table 6 - 17

Median likelihood ratios (LR), calculated from AFP/hCG results and from AFP/hCG/UE3 results for 49 Down's syndrome pregnancies and 390 controls.

	Median LR	
Analytes	Down's syndrome	Controls
AFP/hCG	2.81	0.31
AFP/hCG/UE3	2.42	0.32

assay, while Norgaard-Pedersen *et al.* (1990), using a different immunoassay method, found higher levels of correlation between AFP and UE3 (Controls: r=0.33, Down's: r=0.36). Fisher *et al.* (1989) also noted that AFP and UE3 are not independent in Down's syndrome pregnancies but give no information on the UE3 assay method used. The optimised second trimester RIA kit (Amersham, designated IM4) used in the present study was also used by Spencer *et al.* (1992b) who found levels of correlation (Controls: r=0.31, Down's: r=0.37) similar to those reported here. This suggests that correlation coefficients may be assay dependent.

Reynolds and John (1992) compared the performance of the Amersham third trimester assay (IM2), modified as described by Canick *et al.* (1988), with that of the optimised second trimester kit (IM4), as used in the present study and found significant differences in results. The modified IM2 assay method uses standards with assigned values lower than their true concentration due to the use of a 'zero' standard which contains measurable amounts of UE3. This assay method should thus give lower values in comparison to the optimised second trimester assay method (IM4) which requires no such modification and employs a charcoal stripped zero standard containing no UE3 (personal communication, Dr C Davies, Kodak diagnostics, Cardiff). However consistently higher values were found. These higher values may be indicative of the presence of significant cross-reactivity with other steroids in maternal serum (possibly estetrol and the conjugated forms of estriol). The same study also noted significantly different distributions of UE3 values between the two different assay methods, with an increased spread of MOMs obtained with the optimised second trimester assay.

The UE3 distribution parameters established using the modified IM2 assay cannot be used with the replacement IM4 assay. The low level of correlation and tighter distributions may explain the enhanced performance predicted by Wald *et al.* (1988b) with the addition of UE3 to The AFP/hCG/age combination.

Wald *et al.* (1992a) have predicted an increase in detection of Down's syndrome, at a 5% false positive rate, from 58% to 67% using AFP/hCG/UE3/age when ultrasound is used for dating pregnancies rather than LMP. For AFP/hCG/age the increase was from 54% to 58% using ultrasound estimation of gestation. Of these three analytes, UE3 shows the largest rate of change in median value with gestation between 15 and 20 weeks and hCG the smallest. Interpretation of UE3 results is therefore most affected by inaccuracies in estimates of gestation. In the present study, based on a large screening population in the west of Scotland, around 80% of pregnancies have an ultrasound estimate of gestation available at the time of sampling. Since it is unlikely that in any screening programme all pregnancies will have ultrasound estimates of gestation, the projected gains in detection claimed for UE3 by Wald *et al* (1992a) are unlikely to be realised in practice. Rather a hierarchical system which makes use of the gestational information available at the time of sampling has to be employed in practice.

Considering the four biochemical markers AFP, UE3, hCG and Frβ-hCG individually, hCG and Frβ-hCG are better predictors of Down's syndrome pregnancies than AFP and UE3 (Table 6-14). However, AFP has an important additional role in the detection of pregnancies

at risk for open neural tube defects, and is thus the first choice analyte in any second trimester multiparameter prenatal screening programme, and should be combined with hCG or Frß-hCG.

The benefits of adding UE3 to AFP/hCG/age or AFP/Frß-hCG/age screening for Down's syndrome therefore appear equivocal and careful consideration should be given to the advantages and disadvantages of using a third analyte of marginal predictive value in population screening programmes. For each additional marker assayed, the coefficient of variation of the final risk estimate increases (Holding 1991b, Spencer and Carpenter 1991). Reynolds (1992) estimated that the CV of the overall risk was around 22% when two parameter screening (AFP+hCG) was used and increased to around 40% when three parameter screening (AFP+hCG+UE3) was used. This is borne out by results in the UK External Quality Assessment Scheme for Down's syndrome screening, where the average between-lab variation in risk estimation over six months in 1993 (May-October) for laboratories using AFP/hCG/UE3 (13 centres, CV 53%) is greater than for those using AFP/hCG (30 centres, CV 34%). The cumulative effect of analytical imprecision may ultimately lead to loss of detection in routine clinical practice.

Prospective analysis of AFP, hCG and UE3 in a large series of unselected pregnancies is required to determine the practical value of UE3 as a third analyte for prenatal screening for Down's syndrome.

6.3.2 INTACT VERSUS FREE β hCG IN SCREENING FOR DOWN'S SYNDROME

Recent studies have suggested that Frβ-hCG may be a superior marker to intact hCG in Down's syndrome screening (Macri *et al.*, 1990, Spencer, 1991, Spencer *et al.*, 1992b). Others have predicted a small increase in detection rate for Down's syndrome (Wald *et al.*, 1993a), while others have failed to demonstrate any improvement when using Frβ-hCG (present study, Stone *et al.*, 1993). The summaries of predicted detection rates for Down's syndrome from published studies using intact hCG/age and Frβ-hCG/age (Table 6-14, Section 6.3) suggest that neither marker is significantly better than the other. However, meta-analyses of medians show that the median MOM for Frβ-hCG (2.32 MOM, Table 6-5) is higher than that for intact hCG (2.08 MOM, Table 6-2). However quoted standard deviations tend to be slightly higher in Down's syndrome pregnancies using Frβ-hCG than for intact hCG, with a range of 0.23-0.32 using intact hCG (Table 6-2) compared with 0.27-0.36 using Frβ-hCG (Table 6-5). This increased standard deviation may cancel out the effect of the higher median value found with Frβ-hCG. However one advantage of using Frβ-hCG is that, unlike intact hCG, it is predictive of Down's syndrome at all gestations from 7-20 weeks (Spencer *et al.*, 1992a, Aitken *et al.*, 1993). Prospective analysis of hCG and Frβ-hCG in a large series of unselected pregnancies is required to determine which of these two analytes is the better detector of Down's syndrome in clinical practice in the second trimester of pregnancy.

One question which has been raised as to the usefulness of Frβ-hCG in routine use is the stability of the intact hCG molecule. Since

only 0.5% of total hCG is present as the free ß subunit (Osturk *et al.*, 1988) only a small amount of intact hCG would need to dissociate into α and ß subunits to have a significant effect on the levels of Frß-hCG. Knight and Cole (1991) suggested that Frß-hCG might be artificially elevated in poorly stored samples of maternal serum. Stevenson *et al.* (1993) found that levels of Frß-hCG increased by 14% in 24 hours in whole blood at room temperature, with the rise reaching 43% after four days. Spencer *et al.* (1993) found a slower rate of change, with Frß-hCG levels in whole blood increasing by 2.8% per day at room temperature, and levels 10% higher after 87 hours. Thus increased Frß-hCG levels are likely to be found in blood samples collected from a wide geographical area, which can take several days to arrive at the laboratory. When sample collection and processing time cannot be carefully controlled, a possible alternative would be to collect samples as dried whole blood filter paper spots, which have been shown by Spencer *et al.* (1993) to have no increase in Frß-hCG levels over 9 days when stored at either room temperature or 37°C. Blood spots have been successfully employed in prenatal screening for Down's syndrome (Verloes *et al*, 1992).

In both intact and free ß subunit molecules peptide bonds can be missing between ß subunit residues 44 and 45 or between residues 47 and 48, causing 'nicked' molecules (Sakakibara *et al.*, 1990). There is a greater extent of 'nicking' in the free ß subunit than in intact hCG (Pursieux *et al.*, 1990). Not all Frß-hCG antibodies recognise the 'nicked' form (Kardana and Cole, 1992) and further work is required to establish whether the use of antibodies such as FBT11 which measure total free ß subunit concentration (both

the 'nicked' and 'unnicked' forms) (Kardana and Cole, 1992) give improved sensitivity of detection of Down's syndrome pregnancies.

6.3.4 DIFFERING WAYS OF CALCULATING RISKS FROM ANALYTES

The most common method of calculating risks from analyte levels in the studies discussed in section 6.3 is to use likelihood ratios, which are calculated from the overlapping Gaussian or log Gaussian distributions (Cuckle *et al.*, 1987, Wald *et al.*, 1988a, Wald *et al.*, 1988b, Reynolds and Penney, 1989), and this method has been used in the present study. However some other authors have used differing statistical methods to calculate risks from analyte levels e.g. discriminant analysis (Norgaard-Pedersen *et al.*, 1990, Macri *et al.*, 1990) and a regression model (Ryall *et al.*, 1992).

This Study

This study has compared some of the possible methods of exploiting the predictive value of AFP and hCG for use in screening for Down's syndrome. Wald *et al.* (1988a, 1988b) first suggested a multiparameter likelihood ratio method for combining risks from several variables which has the added refinement of allowing for any correlation between variables. However when this approach was used in the present study to calculate risks from AFP and hCG virtually no difference in detection rates was obtained irrespective of whether correlation was included or not (Table 5-35), with a predicted detection rate for Down's syndrome of 56-57% at a 5% false positive rate.

Arab *et al.* (1988) and White *et al.* (1989) each proposed the use of simple ratio methods for combining AFP and hCG results to provide a combined risk factor, although this approach has been criticized elsewhere (Cuckle *et al.*, 1989a). Using these hCG/AFP ratios alone (Table 5-33), without adding maternal age risks, 57% of Down's syndrome pregnancies had an hCG(MOM)/AFP(MOM) ratio ≥2.5, compared with 5.9% of unaffected pregnancies, very similar to the results of Arab *et al.* (1988). However these results are somewhat at variance with those of Cuckle *et al.* (1989a) although the parameters of the AFP and hCG distributions (means, standard deviations, correlation coefficients) of the latter series and those presented here (Tables 5-2, 5-13, 5-24) are similar. The hCG/AFP ratio method can be extended by combining age risks with the risks derived from the hCG/AFP ratios. This has the effect of decreasing the false positive rate overall, of increasing the detection rate amongst older mothers and of reducing the false positive rate in women under 30 years. A 57% detection rate for Down's syndrome, at a 5% false positive rate, using hCG/AFP ratios combined with maternal age risks, was predicted from retrospective studies (Table 5-35). In routine clinical practice, in 30,084 pregnancies, a detection rate for Down's syndrome of 70% at a 5.1% false positive rate was achieved using this method to calculate risks.

The similarity in detection rates between calculating risks using the separate distributions of AFP and hCG, either with or without correlation (Table 5-35), is to be expected, since the level of correlation found between AFP and hCG in this study is low. The performance of hCG/AFP ratios combined with maternal age risks is

surprising, since this approach theoretically does not give appropriate weight within the calculation to hCG as a better predictor of Down's syndrome than AFP. However the log Gaussian distributions of the Down's syndrome pregnancies and controls are further separated in the hCG/AFP ratios (by 1.64 standard deviations (SD) of the controls) compared with AFP (by 0.83 SD of the controls) or hCG (by 1.34 SD of the controls). The hCG levels in the Down's syndrome pregnancies are contributing more than the AFP levels to this increased separation of the distributions, and therefore the new variable, the hCG/AFP ratio, is weighted according to the respective abilities of hCG and AFP to detect Down's syndrome. Spencer *et al.* (1992b) have found no difference in predicted detection rate for Down's syndrome using either multivariate analysis or hCG/AFP ratios and age, with a 50% detection rate at a 5% false positive rate in both cases. However they did not find this for Frß-hCG, where the use of the ratio method led to a small loss of detection.

Discriminant Analysis.

The predicted detection rates for Down's syndrome obtained by Norgaard-Pedersen *et al.* (1990), using discriminant analysis, are lower at a higher false positive rate for both AFP/hCG/age and AFP/UE3/hCG/age than those obtained by others e.g. present study, Wald *et al.* (1988b) (see Tables 6-15 and 6-16). However the authors attribute this lower detection rate to their lower hCG median in Down's syndrome pregnancies (1.57 MOM), compared with that found in other published studies (see Table 6-2). Macri *et al.* (1990b) used discriminant analysis to calculate risks. Their calculated predicted detection rates for Down's syndrome, both

using Frβ-hCG/age (Table 6-14) or AFP/Frβ-hCG/age (Table 6-15) are the highest when compared with others using the same combination of analytes.

Regression.

Ryall *et al.* (1992) used a regression model to calculate risks for combinations of one to five different analytes from AFP, intact hCG, Frα-hCG, Frβ-hCG, UE3 and hPL, together with maternal age. Detection rates from the regression model are lower than those found in the present study and in other studies (see Tables 6-14, 6-15, 6-16). From their results they concluded that the best detection rate for Down's syndrome might be obtained using five analytes, AFP, Frα-hCG, Frβ-hCG, UE3, and hPL, together with maternal age, and predicted a 64% detection rate at a corresponding false positive rate of 6.8%. To improve the detection rate further, probit analysis was carried out to weight the results of the biochemical variables. This improved the predicted detection rate for Down's syndrome, using the five analytes above, to 76% with a corresponding 3.9% false positive rate. The effect of applying this weighting, derived from probit analysis, to other combinations of analytes was not stated, and it is therefore difficult to compare the effect of this with other methods of calculating risks.

The particular calculation method used to derive risks for all combinations of analytes from published studies is indicated in Tables 6-14, 6-15 and 6-16.

Use of MOMs.

Some authors have cast doubt on the use of MOMs for calculating risks (Macri *et al.*, 1990c, Parvin *et al.*, 1991, Bishop *et al.*, 1993). Macri *et al.* (1990) discussed various factors which may affect calculated MOM values, such as true population differences between centres, assay precision differences and differences in the lower limits of accuracy between assay methods. Parvin *et al.* (1991), using AFP as a model, demonstrate that assay method differences can affect the distribution of MOMs and that the impact is greater on the lower tail of the distribution than on the upper tail, due to the log Gaussian distribution of AFP. Disparities in risk estimates are less likely to arise if laboratories use distribution parameters for each analyte for the affected and unaffected populations that are based on the local assay method. Laboratories should check the distributions of MOM in their own pregnant population before applying published risk tables or calculation packages.

Bishop *et al.* (1993) demonstrate that there are differences in percentiles above and below different threshold MOMs at different gestations in a model population. The differences appeared less marked when applied to a data base of 5,423 actual patient AFP results between 15-19 weeks. The authors question the validity of combining data in MOM from different gestational ages to calculate risks. However they do not suggest an easily applied alternative and the differences found in an actual patient data set are small.

6.4 FACTORS AFFECTING THE INTERPRETATION OF SERUM MARKER RESULTS

6.4.1 GESTATION

Interpretation of screening results is critically dependent on an accurate knowledge of the gestation at the time of sampling. The gestation used affects the conversion of concentration units for the various serum markers into MOM from which likelihood ratios are derived. In cases where gestation is over-estimated hCG and Frß-hCG results will be higher than expected and AFP and UE3 results lower. This is because at 12-14 weeks gestation hCG and Frß-hCG results are higher and AFP and UE3 results lower than at 15-20 weeks. Underestimated gestation will therefore cause risks to be underestimated.

In the retrospective studies on serum markers presented here complete information on gestation either by certain dates (LMP) or by ultrasound estimation was not available for all samples. Around 80% of pregnancies had an ultrasound estimate of gestation available at the time of sampling, and a slightly smaller proportion had information both from LMP and ultrasound estimation, which in many cases was discordant. Therefore a hierarchical system to select the most appropriate gestation was devised. Estimates of gestation were based mainly on the time since the first day of the last menstrual period (LMP), modified by ultrasound as suggested by Rossavik and Fishburne (1989). Ultrasound estimation of gestation was used only when the date of the LMP was not available or there was a discrepancy of two weeks or greater between the gestation estimated from the LMP and that

from ultrasound examination. The gestations used therefore reflect the quality of information likely to be available in routine practice and avoids dependence on a single dating method which is unlikely to be available for all samples.

The above system of determining gestation has been used successfully in routine prospective screening with an additional modification to take into account of whether LMP information was classified as 'certain' or 'uncertain'. Where LMP information was uncertain, an ultrasound estimate of gestation, if available, was used in preference. Computer software is readily available to recalculate Down's syndrome risks for patients who have an ultrasound scan to review gestation after the test results have been sent back to the referring hospital. However it should be noted that the policy of reviewing gestation by ultrasound after the report of a positive screening result, which has the effect of removing a proportion of women from the high risk group may ultimately lead to loss of detection of Down's syndrome pregnancies. It is less frequent for women who are assigned to the low risk group to have reviewed the gestation on which their original risk calculation was based.

Wald *et al.* (1992a), in a study of over 2,000 women having routine screening, predicted that use of ultrasound estimates of gestation, rather than those based on LMP, would increase the detection rate for Down's syndrome, at a 5% false positive rate for AFP/hCG/age from 54% to 58%, and for AFP/hCG/UE3/age from 58% to 67%. Holding (1991a) estimated that if solely LMP information was available at the time of screening, around 15% of women would

need to have a recalculation of risk. Both Holding (1991a) and Gardosi and Mongelli (1993) have proposed that results from screening programmes be reported for the range of gestations within the screening period.

6.4.2 TWINS

In the present study maternal serum AFP and hCG levels in 81 twin pregnancies were, on average, approximately double those found in singleton pregnancies, with a median hCG level of 1.85 MOM and a median AFP level of 1.91 MOM. In one twin pregnancy discordant for Down's syndrome an hCG level of 4.63 MOM and an AFP level of 1.94 MOM were found.

Similar results in unaffected twin pregnancies have been reported by others. Alpert *et al.* (1990), in 51 twin pregnancies, found AFP, hCG and UE3 medians of 1.58 MOM, 1.80 MOM and 1.44 MOM respectively. Canick *et al* (1990b), in 35 twin pregnancies, found levels of 2.32 MOM, 1.93 MOM and 1.67 MOM respectively and Wald *et al.* (1991), in 200 twin pregnancies, 2.13 MOM, 1.84 MOM and 1.67 MOM respectively. Neibiolo *et al.* (1991) found median hCG levels of 2.26 MOM in twin pregnancies. The UE3 levels in twin pregnancies are lower than those found for AFP or hCG.

There is little available data on serum marker levels in twin pregnancies concordant or discordant for Down's syndrome. Wald *et al.* (1991) have proposed a screening policy for twins, using median values for twins to calculate MOMs and from these

calculating Down's syndrome risks using the parameters of affected and unaffected singleton pregnancies. This would be expected to yield a similar false positive rate for Down's syndrome to that for singleton pregnancies but the detection rate would be expected to be lower because of the presence of an unaffected co-twin. Information on marker levels in twin pregnancies in which one or both twins have Down's syndrome needs to be collected, perhaps by means of a multicentre study, before the likely predictive value of this approach can be properly assessed and before this type of screening is implemented.

Screening for Down's syndrome in twin pregnancies presents a clinical dilemma. Invasive diagnostic procedures in twin pregnancies are more difficult and in cases where a Down's syndrome fetus is diagnosed the presence of an unaffected co-twin raises the problem of the coincidental termination of a normal fetus or the medical complication of selective feticide. Therefore reporting Down's syndrome risks in twin pregnancies may not be readily accepted by obstetrician or patient.

6.4.3 THREATENED ABORTION

Pregnant women experiencing vaginal bleeding have elevated levels of maternal serum AFP more often than the general pregnant population (Wald *et al.*, 1977, Lidbjork *et al.*, 1977). This is thought to reflect feto-maternal haemorrhage in these cases, with the elevated AFP levels originating from fetal blood, in which the AFP concentration is around 50,000 times higher than in the

maternal circulation at 17 weeks gestation (Wald and Cuckle, 1984).

The present study has found unchanged levels of maternal serum hCG in cases of threatened abortion, even in those cases with elevated AFP. Levels of hCG in the fetal circulation are lower than those found in the maternal circulation (Vaitukaitis, 1977), by a factor of between 100 and 1000 times (Gordon and Chard, 1979), and feto-maternal haemorrhage is therefore unlikely to influence maternal serum hCG levels.

Episodes of bleeding, particularly if recently proceeding the collection of the screening blood sample, may cause a rise in maternal serum AFP levels, and there will therefore be a tendency for risks to be underestimated in case of threatened abortion. Since hCG levels are unlikely to be affected it is possible in these cases to calculate a risk of Down's syndrome using hCG/age only, with around a 6% loss of predicted detection for Down's syndrome.

6.4.4 INSULIN DEPENDENT DIABETES MELLITUS

The present study found slight lowering of both AFP and hCG levels in maternal serum, to 0.94 MOM and 0.90 MOM respectively, in 56 pregnancies in woman affected by IDDM. The similar lowering of both AFP and hCG will almost cancel each other out when calculating Down's syndrome risks, and in this study (Section 5.3.1.4) the diabetic pregnancies where neither over- nor

under-represented in the high risk groups for Down's syndrome or neural tube defects.

Similar levels of AFP and hCG in 24 IDDM patients were found by Canick *et al.* (1990), with levels of 0.97 MOM and 0.87 MOM respectively. UE3 levels were 0.87 MOM. Wald *et al.* (1992c), in samples taken from 92 diabetic pregnancies between 1975 and 1983, found similar levels of hCG to the present study and that of Canick *et al.* (1990), with a median level of 0.95 MOM. The UE3 level in the diabetic pregnancies was 0.92 MOM. However the AFP levels were lower than those found in the present study and by Canick *et al.* (1990) with a median value of 0.77 MOM, but less reduced than those previously reported from the same centre (0.60 MOM, Wald *et al.*, 1979). Reece *et al* (1987) found similar AFP levels to those found in the present study in 39 IDDM patients, who had a median AFP levels of 0.91 MOM (0.96 MOM when weight corrected). The level of AFP found by Baumgarten *et al.* (1988), in 46 IDDM patients was 0.8 MOM.

There appears to be a difference in AFP levels in IDDM patients from recent samples, such as in this study, from those found by Wald *et al.* (1992c) in samples taken more than a decade ago. This difference may reflect improving control of IDDM in pregnancy. Several studies have used high levels of glycosylated haemoglobin as a marker for poor control of IDDM. Reece *et al.* (1987) found an inverse correlation between glycosylated haemoglobin and AFP in 161 IDDM patients, for samples taken within six weeks of each other. However Powrie *et al.* (1987), in 27 IDDM patients, did not find this effect. Baumgarten and Robinson (1988), in 46 IDDM

patients, confirmed the findings of Reece *et al.* (1987) with the patients with the highest levels of glycosylated haemoglobin having the lowest levels of AFP. Martin *et al.* (1990) found that the 25% of the 93 pregnancies in their study who had levels of glycosylated haemoglobin greater than 4 SDs above the normal mean had a lower AFP level (0.68 MOM) when compared with the remaining 75%, who had a mean AFP level of 0.84 MOM.

Caution should therefore be applied when using correction factors for AFP results in IDDM patients. Published correction data based on patient samples taken more than a decade ago (e.g. Wald *et al.* 1979, 1992c) would appear to be inappropriate to currently pregnant diabetic patients and their use might lead to loss of detection of Down's syndrome pregnancies.

6.4.5 MATERNAL WEIGHT

The present study has found that women of greater than average maternal weight tend to have lower than average serum concentrations of AFP and hCG, while lighter than average women have increased concentrations. The effect of maternal weight on AFP levels had been previously noted ((Haddow *et al.*, 1981, Wald *et al.*, 1981, Crandall *et al.*, 1983), and various formulae proposed for correcting AFP values for maternal weight (Wald *et al.*, 1981, Johnston and Lingley, 1984, Palomaki *et al.*, 1985). Drugan *et al.* (1989b) have pointed out that obese women have a greater relative increase in body fat, and hence a smaller actual plasma volume relative to weight than women of average weight.

Because of this formulae for weight correction may cause an overcompensation for weight in women over 90 Kg. When screening using AFP/age, correction for maternal weight will affect the risks calculated for individual women, but will have minimal impact on overall screening performance.

Other studies have shown the same trends for AFP, hCG and UE3 (Palomaki *et al.*, 1990, Wald *et al.*, 1992a, Bartels *et al.*, 1993). When AFP and hCG are used together, either with or without UE3, maternal weight has less effect on the calculation of risks for individual women, and correcting for maternal weight will have virtually no effect on overall screening performance in terms of detection rate for Down's syndrome and corresponding false positive rate (present study, Palomaki *et al.*, 1990, Wald *et al.*, 1992a).

6.4.6 RACE

The median levels of AFP, hCG and UE3 found in black women, expressed as multiples of the median of the levels in Caucasian women, from published studies, are shown in Table 6-18. Levels of AFP found are 10-13% higher in black women. For hCG a range of levels was found in black women, with an average 12% increase over the hCG level found in Caucasian women. However, two studies found no change in levels in black women (Petrocik *et al.*, 1989, Canick *et al.*, 1990) while three others found levels which were between 19-27% higher (Muller and Boué, 1990, Simpson *et al.*, 1990, Kulch *et al.*, 1993). One study (Bogart *et al.*, 1991) found a higher

Table 6 - 18

Levels of AFP, hCG and UE3 found in black women, expressed as multiples of the median for Caucasian women, from published studies

	Weight Corr.	n	AFP (MOM)	hCG (MOM)	UE3 (MOM)
Crandall *et al.* (1983)	Yes	288	1.10	-	-
Macri *et al.* (1987)	No	1,954	1.13	-	-
Petrocik *et al.* (1989)	No	50	-	1.00	-
Canick *et al.* (1990)	No	235	1.12	1.03	0.95
Muller & Boué (1990)	No	214	-	1.27	-
Simpson *et al.* (1990)	No	300	-	1.21	0.95
Bogart *et al.* (1991)	No	310	-	1.00	-
	Yes		-	1.10	-
Kulch *et al.* (1993)	Yes	134	-	1.19	1.05

levels in black women only when the results were weight corrected. Black women tend, on average, to be heavier than Caucasian women (Crandall *et al.*, 1983, Bogart *et al.*, 1991). UE3 levels appear unchanged in black women.

Crandall *et al.* (1983) found no difference in AFP levels between either Hispanic or Oriental women and Caucasian women. Bogart *et al.* (1991) found weight corrected hCG levels 16% higher in a small series of Oriental women, but no statistical difference in levels between Hispanic and Caucasian women.

For screening populations with significant numbers of black women or other ethnic groups risks should be calculated after correction of AFP and hCG results, by using medians appropriate to the particular ethnic group.

6.4.7 MATERNAL SMOKING

It has been demonstrated by Palomaki *et al.* (1993) and Bartels et al. (1993) that in pregnant women who smoke, levels of hCG were reduced to around 80% of the levels in non-smoking women. Levels of AFP and UE3 were virtually unchanged between smokers and non-smokers. Recent unpublished results from this laboratory would confirm this, with a hCG median of 0.80 MOM in 617 smokers, compared with 1.13 in 1514 non-smokers. For AFP the median levels were 1.04 MOM in the smokers and 0.98 MOM in the non-smokers. The smokers were significantly lighter (difference in medians: 1.7 Kg) and younger (difference in medians: 4 years). The lower hCG

results will cause smokers to be under-represented in the high risk group for Down's syndrome compared with non-smoking women of the same age and the use of different hCG median values for smokers and non-smokers could be considered. There is however no information currently available on whether levels of hCG are similarly lowered in smoking women carrying a Down's syndrome fetus.

Some published studies have found a deficit of smokers among women who had pregnancies associated with Down's syndrome (Hook and Cross, 1985, 1988, Christianson and Torfs, 1988). Cuckle et al (1990b) found a lower, but not statistically significantly different, proportion of smokers amongst women who had a pregnancy associated with Down's syndrome, compared with age matched controls. Data on Down's syndrome rates in smokers and non-smokers need to be interpreted with caution, since pregnant women who smoke tend to be younger than those who do not, and therefore on a population basis the Down's syndrome incidence will tend to be lower in smokers, purely on the basis of age. Further studies, carefully controlled for age are required to determine whether smoking does have a protective effect against a Down's syndrome birth.

6.4.8 REPEAT TESTING

Because of regression to the mean, a second sample will tend to have pregnancy marker results which are closer to the average value than those for the first sample (Haddow *et al.*, 1986). Cases

affected by Down's syndrome are as likely as unaffected pregnancies to be moved from the high risk group to the low risk group by the results from a second sample. Repeat testing (except in cases where the gestational has shown that the first sample has been taken too early) is inappropriate and will lead to loss of detection of affected cases. Lustig *et al.* (1988), in the California AFP screening programme for Down's syndrome, reported five out of 23 cases of Down's syndrome which had not been detected, due to repeat testing reassigning them from the high to the low risk group.

Cuckle *et al.* (1989b), although considering repeat testing an unjustified policy, compiled a nomogram to combine AFP results from the first and second sample to give a Down's syndrome risk combined from both. To combine results of multiple markers from first and second samples in this way would be considerably more complex.

In the west of Scotland prenatal screening programme, repeat testing of women who are assigned to the high risk group for Down's syndrome is discouraged. However where a repeat sample is received, and the results of the second assign the patient to the low risk group, an additional comment is added to the report, stating that this does not invalidate the Down's syndrome risk from the first sample.

6.5 SCREENING FOR DOWN'S SYNDROME IN ROUTINE CLINICAL PRACTICE

Screening protocols for the detection of chromosomally abnormal pregnancies, developed from retrospective studies of pregnancy markers in stored maternal serum samples, are increasingly being applied in clinical practice. There is wide variation in the combinations of markers used and in the age distributions of the populations in which they are applied, and this is reflected in the variations in detection and false positive rates reported in many studies. Care is therefore required when comparing the performance of different screening programmes.

6.5.1 AFP/AGE SCREENING IN PROSPECTIVE USE

Data derived from the investigation of AFP levels in pregnancies with autosomal trisomy in the present study (Section 5.1) were used to modify the existing maternal serum AFP screening programme for NTD operating in the west of Scotland since 1976, to provide risks of an autosomal trisomy in individual pregnancies.

Over a 3½ year period approximately 100,000 pregnancies were screened using this protocol and a detection rate for Down's syndrome of 41% was obtained, with a false positive rate of 6.1%. The detection rate for all autosomal trisomies was 43% (Tables 5-9, 5-10). However some older women were offered amniocentesis or chorionic villus sampling solely on the basis of age ≥35 years either preceding or instead of a maternal serum screening test and the autosomal trisomy pregnancies diagnosed in this way are shown

in Table 5-11.

Some difficulties emerged in the application of this type of screening. Interpretation of numerical risks proved problematical for both patients and medical staff who were more familiar with the simpler concept of a maternal age threshold. The rate of diagnostic testing was significantly higher in older women than in younger women with the same calculated risk although in both cases the calculated risks are exactly the same and the age factor already incorporated. Although uptake of diagnostic testing overall was 42% and therefore the potential detection rate for autosomal trisomies of 43% was reduced to an actual prenatal diagnosis rate of 25% (Table 5-10), this figure represents a substantial increase in detection over the original system based solely on maternal age. Thus although AFP is a relatively weak marker for Down's syndrome, when combined with maternal age and applied in whole population screening it provides a significantly better method of utilizing prenatal diagnostic resources.

Data from other published prospective studies are summarised in Table 6-19. All of these other studies (DiMaio *et al.*, 1987, Lustig *et al.*, 1988, New England Collaborative Study, 1989) had incomplete ascertainment of Down's syndrome cases, with the number of cases in the women assigned to the high risk group being estimated from the age distribution of the screened population. Also these studies differed from the west of Scotland study described above in that all three were confined to women who were all or mainly under 35 years of age. However comparison of all four studies, including this one, shows an approximately linear

Table 6 - 19

Comparison of detection rates for Down's syndrome and corresponding false positive rates achieved using AFP/age screening in routine practice.

	No.women screened	False positive rate	Detn rate
Palomaki (1986)	51,141	2.1[ac]	21[acd]
Di Maio *et al*, (1987)	34,354	4.2[bd]	33[bd]
Lustig *et al*. (1988)	174,784	2.3[a]	21[ad]
New England collaborative study (1989)	77,273	2.7[a]	25[ad]
Present study	100,481	6.1	41

[a] Women aged <35 years only.

[b] Mainly women aged <35 years.

[c] Cases also included in New England collaborative study (1989).

[d] Total number of Down's syndrome pregnancies in screened population estimated.

increase in detection with increasing false positive rate. All demonstrate that is possible to extend screening for Down's syndrome to women younger than 35 years by using AFP/age rather than maternal age alone. Down's syndrome cases are detected in these younger women which would not otherwise have been prenatally diagnosed if age had been the only criterion on which to select women for diagnostic testing.

6.5.2 MULTIMARKER/AGE SCREENING IN PROSPECTIVE USE

Analysis of hCG was added to the existing west of Scotland AFP screening programme in September 1991. Up to September 1992 30,084 pregnancies were screened (See Section 5.3.2) and 70% of Down's syndrome pregnancies in these women assigned to the high risk group. The uptake of diagnostic testing by women in the high risk group increased to 70%, in women of all ages, resulting in 56% of the Down's syndrome pregnancies in the screened population being prenatally diagnosed. Addition of hCG analysis to the existing screening programme has resulted in a marked increase in the prenatal diagnosis rate for Down' syndrome.

Data from published prospective studies using two analytes together with maternal age are summarised in Table 6-20 and for three analytes and maternal age in Table 6-21. The three studies using double analytes (Herrou *et al.*, 1992, Spencer and Carpenter, 1993, present study), with a total of over 48,000 pregnancies, have a higher detection rate than three of the four studies using triple analytes, (Wald *et al.*, 1992, Haddow *et al.*, 1992, Phillips

Table 6 - 20

Comparison of data from published prospective Down's syndrome screening studies using two markers in addition to maternal age. DS = Down's syndrome, HRG = High risk group.

	Herrou *et al.* (1992)	Spencer & Carpenter (1993)	Present study
Analytes used	UE3/hCG	AFP/Frβ-hCG	AFP/hCG
No.women screened	10,000[a]	8,179	30,084
% ≥35 years	-	10.6%	6.9%
% ≥37 years	0%	4.9%	3.0%
Inital false +ve rate	-	6.9%	6.3%
Final false +ve rate	4.7%	5.2%	5.1%
No. of women in HRG	466	426	1,523
No.DS in screened population	10	16	37
No. DS detected	6	11	26
Detection rate (95% CI)	60% (26-88)	69% (41-89)	70% (53-84)
Uptake of diagnostic testing	88%	89%	70%
DS rate/1000	1.0	2.0	1.2
Overall odds of DS if in HRG	1:47	1:39	1:59

[a] All women aged <38 years.

Table 6 - 21

Comparison of data from published prospective Down's syndrome screening studies using three markers in addition to maternal age. DS = Down's syndrome, HRG = High risk group.

	Wald *et al.* (1992b)	Haddow *et al.* (1992)	Phillips *et al.* (1992)	Cheng *et al.* (1993)
Analytes used	AFP/hCG/ UE3	AFP/hCG/ UE3	AFP/hCG/ UE3	AFP/hCG/ UE3
No.women screened	12,603	25,207	9,530[a]	7,718
% ≥35 years	-	4.9%	0%	10.7%
% ≥37 years	4.8%	-	-	-
Inital false +ve rate	5.7%	6.6%	7.2%	8.0%
Final false +ve rate	4.1%	3.8%	3.2%	6.0%
No. of women in HRG	514	962	307	461
No.DS in screened population	25	36	7	22
No. DS detected	12	21	4	20
Detection rate (95% CI)	48% (28-69)	58% (41-74)	57% (18-90)	90% (71-99)
Uptake of diagnostic testing	75%	79%	70%	69%
DS rate/1000	2.0	1.4	0.7	2.9
Overall odds of DS if in HRG	1:43	1:46	1:77	1:23

[a] Women aged <35 years only.

et al., 1992).

However, each of the double marker plus maternal age studies used different combinations of analytes. Herrou *et al.* (1992), in western France, used a combination of hCG/UE3/age in women aged less than 38 years. The detection rate for Down's syndrome was 60% at a 4.7% false positive rate, but would have been expected to be higher if women of all ages had been screened. Spencer and Carpenter (1993), in Romford, Essex, used a combination of AFP/Frß-hCG/age applied to the whole pregnant population regardless of age, and achieved a detection rate for Down's syndrome of 69% at a false positive rate of 5.2%. The present study, in the west of Scotland, in a whole pregnant population, had a detection rate for Down's syndrome of 70% at a 5.1% false positive rate. In routine use in the second trimester, comparing the west of Scotland data with that of Spencer and Carpenter (1993), there appears little difference in detection of Down's syndrome when either intact or free ß subunit hCG is used in combination with AFP and age. Herrou *et al.* (1992) used UE3 instead of AFP and achieved approximately the same rate of detection for Down's syndrome, suggesting that UE3 is an effective substitute for AFP. However AFP is also used for screening for neural tube defects and this would be lost if UE3 were used instead.

Four prospective studies using the three markers, AFP, UE3 and hCG have been published (Wald *et al.*, 1992b, Haddow *et al.*, 1992, Phillips *et al.*, 1992, Cheng *et al.*, 1993). These achieved detection rates for Down's syndrome ranging from 48-91% at false

positive rates of between 3.2-6.0%. The most representative population with regard to age distribution was that screened by Wald *et al.* (1992b) in which 4.8% of women were aged 37 years and over. A detection rate for Down's syndrome of 48% was obtained at a false positive rate of 4.1%. Haddow *et al.* (1992), in New England, USA reported a detection rate for Down's syndrome of 58% at a false positive rate of 3.8% in a screened population where only 4.9% of women were aged 35 years and over. Phillips *et al.* (1992), in Tennessee, screened only women aged less than 35 years and achieved a detection rate for Down's syndrome of 58% at a 3.2% false positive rate. The detection rate (and corresponding false positive rate) in these last two studies would have been expected to be higher if all older pregnant women had also been screened. The fourth study (Cheng *et al.*, 1993), in Seattle, reported a detection rate for Down's syndrome of 91% at a false positive rate of 6.0%. The screened population in this study appears atypical as the incidence of Down's syndrome is much higher (2.9/1000) than would be expected in a typical pregnant population, and also when compared with the other studies in Tables 6-20 and 6-21. This may be attributed to the skewed age distribution, with only 19.8% of women aged less than 25 years, compared with 39% in the west of Scotland pregnant population, and 35.9% aged 30-34 years compared with 23.8% in the west of Scotland.

These studies, including the data presented here in section 5.3.2, demonstrate that the use of multiple markers in combination with maternal age to screen for Down's syndrome in the second trimester is an effective strategy in clinical practice. However there is no evidence to suggest that any of the different marker combinations

used (AFP/hCG, AFP/Frß-hCG, UE3/hCG or AFP/hCG/UE3) offers significantly better or worse detection and false positive rates than any of the others. Meaningful comparisons of detection performance are however only possible in large populations of comparable age distributions and at standardised false positive rates.

6.6 BIOCHEMICAL SCREENING FOR TRISOMY 18

6.6.1 AFP SCREENING FOR TRISOMY 18

The present study predicted that, using AFP/age screening and a cut-off risk of 1:280, 37% of autosomal trisomy pregnancies, which will include trisomy 18 pregnancies, would be assigned to the high risk group. This has been borne out in routine practice. In 100,481 pregnancies screened 7 of 13 (54%) trisomy 18 pregnancies were detected by screening (Table 5-10). No other investigators appear to have attempted to optimise the detection of autosomal trisomies other than Down's syndrome in AFP/age screening programmes by using appropriately modified age risks and AFP distribution data as described in this study in Section 5.1. Drugan *et al.* (1989a), reviewed the results of cytogenetic analyses performed on 1154 pregnancies when the indication was low maternal serum AFP, and found 6 Down's syndrome pregnancies, 1 trisomy 18 pregnancy, and 6 other chromosome abnormalities. They concluded that risks quoted by screening programmes should include other chromosome abnormalities.

Reviewing other published prospective series of AFP/age screening shows that programmes set up to detect Down's syndrome also detect a proportion of trisomy 18, and other chromosome abnormalities. Di Maio *et al.* (1987) found three trisomy 18 and one trisomy 13 pregnancies in addition to nine Down's syndrome pregnancies within a high risk group of 1451. Lustig *et al.* (1998), in the 1940 women who opted for amniocentesis out of 2552 women in the high risk group, found four trisomy 18 and one trisomy 13 pregnancies in addition to 17 Down's syndrome pregnancies. The New England Collaborative Study (1989) found four trisomy 18 pregnancies in addition to 18 Down's syndrome pregnancies in the 1593 women in the high risk group who opted for amniocentesis.

6.6.2 MULTIMARKER SCREENING FOR TRISOMY 18

The highly significant reduction in maternal serum hCG levels found in trisomy 18 pregnancies (Section 5.2.1.1.3) offers a further opportunity to detect trisomy 18 pregnancies through selection of a second high risk group.

Using the protocol based on the analysis of AFP and hCG in 12 trisomy 18 and 7830 unaffected pregnancies in this study, a detection rate of 67% was predicted for additional false positive rate of just over 1%. However routine screening in 30,084 women detected only two out of seven (29%, 95% CI 4-71%) of trisomy 18 pregnancies within the high risk group of 0.3%. With this small number of trisomy 18 pregnancies the confidence interval is wide and greater numbers are required to properly assess the likely

success of this screening policy. Once AFP and hCG results have been accumulated from a sufficiently large number of trisomy 18 pregnancies (e.g. greater than 25) it should be possible to assign to women an exact risk of trisomy 18, using likelihood ratios derived from the distribution data.

Other authors have, from retrospective studies, described varying protocols for screening for trisomy 18. Canick *et al.* (1990a), using results of 10 cases of trisomy 18, proposed that a simple cut-off system, taking samples which had AFP results ≤0.75 MOM, UE3 results ≤0.60 MOM and hCG results ≤0.55 MOM, would detect 60% of trisomy 18 pregnancies, with a corresponding false positive rate of 0.4%.

Miller *et al.* (1991) reported that four of nine (44%) trisomy 18 pregnancies had hCG levels less than 0.25 MOM, and that less than 1% of unaffected pregnancies had values below this level.

Staples *et al.* (1991), using a similar approach to that which they have proposed for Down's syndrome screening (Ryall et al., 1992), devised a regression equation, using results from 12 cases of trisomy 18 pregnancies and 390 controls. They predicted a 58.3% detection rate for trisomy 18, with a corresponding false positive rate of 0.3%, using a combination of five analytes, Frβ-hCG, UE3, estradiol, Frα-hCG,and hPL. Using only Frβ-hCG and UE3 gave a similar predicted detection rate of 57.9%, at the same false positive rate.

Barkai *at al.* (1993) combined data from their own series of 15

trisomy 18 pregnancies with that from other published studies, and by meta-analysis derived the parameters for log Gaussian distributions for AFP, UE3 and hCG. Using these together with maternal age risks for trisomy 18 (taken to be one tenth of those for Down's syndrome compiled by Cuckle *et al.* (1987)), they estimate that 67% of trisomy 18 pregnancies might be detected, with a false positive rate of 0.3%.

Palomaki *et al.* (1992) applied prospectively the protocol that they had previously proposed (Canick *et al.*, 1990a) to 19,491 women screened. Of these 92 women (0.5%) were assigned to the high risk group and six cases of trisomy 18 were identified. However the number of trisomy 18 pregnancies in the whole screened population was not available, but was estimated from the age distribution to be seven, giving an estimated detection rate of 85%.

Thus, screening for trisomy 18 using a variety of different protocols appears to be a practical proposition, with an acceptably small percentage of women additionally being identified at high risk. The best markers appear to be either intact hCG or Frß-hCG and UE3.

6.7 IMPACT OF BIOCHEMICAL SCREENING FOR DOWN'S SYNDROME IN THE WEST OF SCOTLAND

In the west of Scotland the introduction of biochemical screening methods has led to increasing prenatal detection of Down's

syndrome. Figure 6-1a compares the false positive rates in the screened population obtained using maternal age alone, AFP/age screening and hCG/AFP/age screening. The corresponding uptake of diagnostic testing is also shown. Figure 6-1b shows the corresponding detection rates and prenatal diagnosis rates for the different screening methods. It can be seen that the false positive rate and uptake of diagnostic testing were similar for age screening and AFP/age screening, but the detection rate increased by over 30% and the prenatal diagnosis rate almost doubled to 23% with the introduction of AFP/age screening. With the addition of hCG analysis the false positive rate fell by over 15% from 6.1% to 5.1%, but an increased number of women (3.6% vs 2.6%) had diagnostic testing. The detection rate increased further to 70% with 56% of affected cases in the screened population being prenatally diagnosed. AFP/hCG/age screening has more than doubled the detection rate for Down's syndrome over that achievable with maternal age alone, and, because of the increased uptake of diagnostic testing by women in the high risk group, has given a fourfold increase in the number actually prenatally diagnosed.

The impact of various screening measures applied in the west of Scotland on the birth incidence of Down's syndrome is presented in Figure 6.2. This takes into account women who proceeded directly to diagnostic testing without participating in screening, the incomplete uptake of diagnostic testing by women at increased risk and also the proportion of women who decline prenatal screening (20%). In 1986, when age was used to select women for diagnostic testing, 13% of Down's syndrome pregnancies were detected prenatally. For 1987-1990, when AFP/age screening was routine and

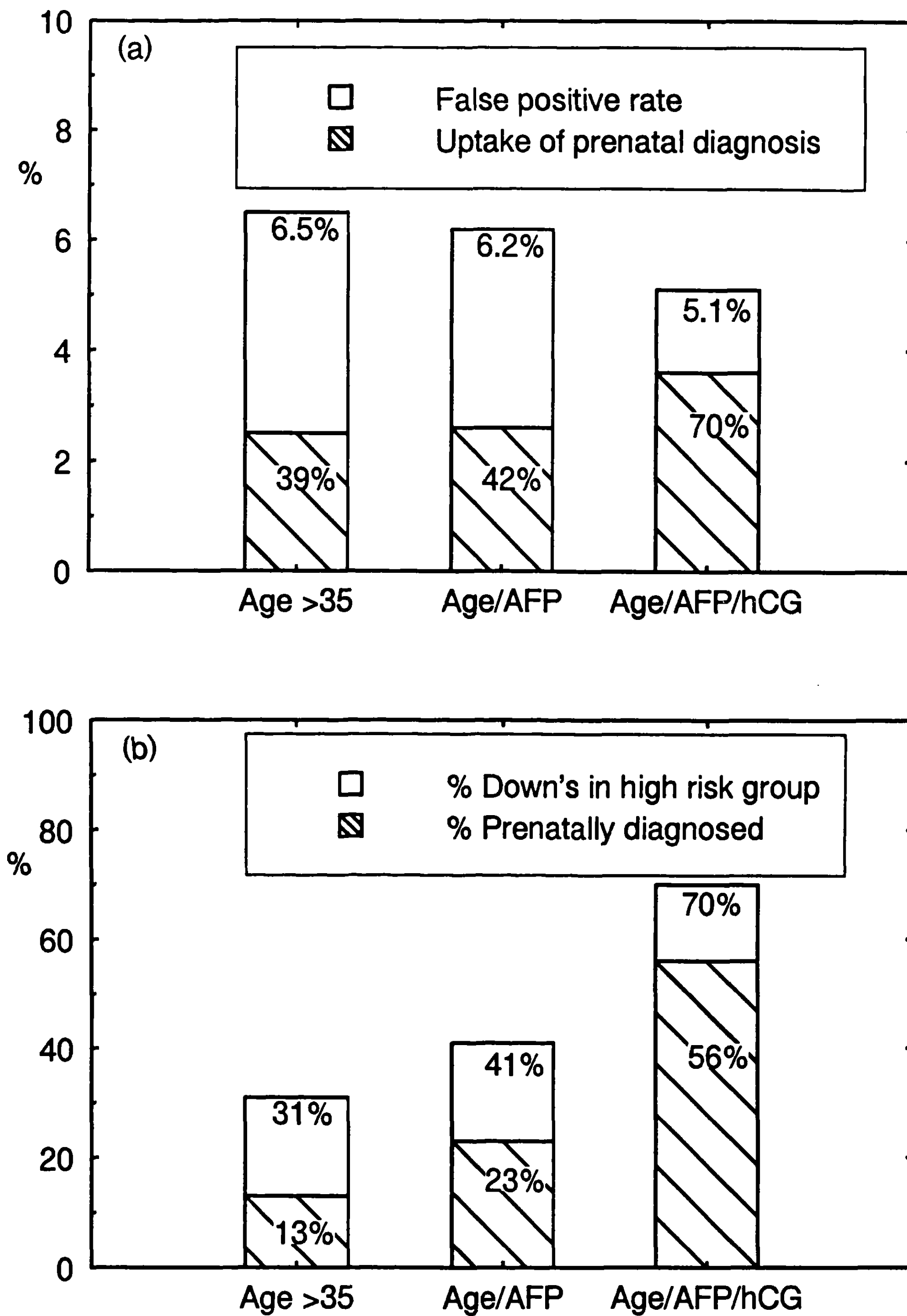


Figure 6 - 1

Screening in the west of Scotland using age (1986), age/AFP (July 1987-Dec 1990), age/AFP/hCG (Sept 1991-Sept 1992).

(a) False positive rates and corresponding uptake of diagnostic testing.

(b) Detection rates for Down's syndrome and corresponding prenatal dignosis rates.

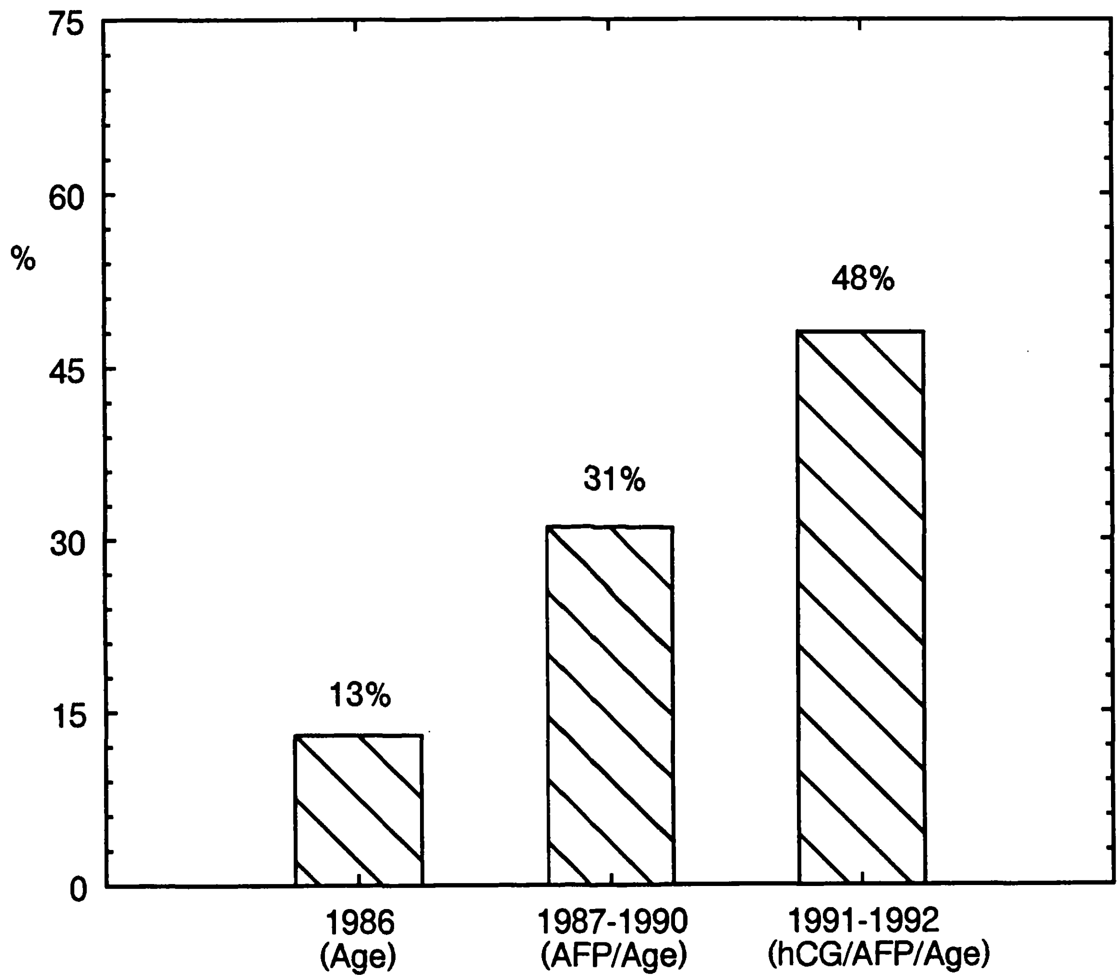


Figure 6 - 2

Proportions of Down's syndrome pregnancies prenatally diagnosed in the west of Scotland in 1986 (age screening), July 1987 - Dec 1990 (AFP/age screening) and Sept 1991 - Sept 1992 (hCG/AFP/age screening).

some additional cases were detected using maternal age criteria, detection increased to 31%. The introduction of hCG analysis in 1991 resulted in a further increase in prenatal detection of Down's syndrome, to 48% of all affected pregnancies, a total which includes some cases diagnosed prior to screening by CVS in the first trimester. Thus almost four times as many Down's syndrome pregnancies are now prenatally diagnosed with hCG/AFP/age screening compared with that achieved by maternal age. AFP/hCG/age screening has also improved the prenatal diagnosis rate in women aged 35 years and over, from 42% in 1986 to 69% in September 1991 - September 1992 through increased compliance of diagnostic testing.

The displacement of maternal age screening by multimarker biochemical screening has extended testing to the whole pregnant population, irrespective of age, and identifies a proportion of younger women as being at increased risk. Many women aged ≥35 years are now given a reduced risk and are spared the necessity for diagnostic testing, with its known risk of miscarriage.

It is known that considerable anxiety may be generated by a positive screening result (Abuelo *et al.*, 1991, Keenan *et al.*, 1991), and much effort is required to convey to women the rationale of screening before they decide to be screened. Counselling should include an explanation of the type of abnormalities being screened for, the meaning of being screen positive or screen negative and the possible need for follow-up tests. To help towards this end the west of Scotland screening programme has produced a leaflet explaining the test to patients,

and also a booklet for ante-natal clinic staff which gives more information about the test and further detail about such things as detection rates for Down's syndrome in different age groups and explanations of typical report comments.

The options following a positive diagnostic results should also be explained and counselling should be available for women who choose to terminate or choose to continue an affected pregnancy. Continued liaison between laboratories providing a screening service and the obstetric units is required so that developments and improvements to screening protocol may be incorporated into routine practice without adverse affect.

6.8 FUTURE PROSPECTS

The current timing of prenatal screening for Down's syndrome is dictated by the need to carry out screening for neural tube defects at around 16-18 weeks gestation when maximum sensitivity is achieved. Neural tube defect pregnancies do not have elevated levels of maternal serum AFP in the first trimester of pregnancy (UK Collaborative Study, 1977, Aitken *et al.*, 1993). However there is mounting evidence that maternal serum screening for Down's syndrome may be possible in the first trimester, which would bring the benefit of earlier detection and termination of pregnancy. Retrospective studies have identified several markers which show variation in Down's syndrome pregnancies at this stage of gestation, although the pattern of variation is different for some markers between the first and second trimesters.

In first trimester Down's syndrome pregnancies levels are reduced for AFP (Brambrati *et al.*, 1986, Barkai *et al.*, 1987, Brock *et al.*, 1990, Wenger *et al.*, 1990, Van Lith., 1991), UE3 (Cuckle *et al.*, 1988, Brock *et al.*, 1990, Crandall *et al.*, 1991, Aitken *et al.*, 1993) PAPP-A (Brambrati *et al.*, 1993, Wald *et al.*, 1993b, Muller *et al.*, 1993) and SP_1 (Brock *et al.*, 1990, MacIntosh *et al.*, 1993). Frß-hCG levels are markedly elevated (Macri *et al.*, 1993, Aitken *et al.*, 1993) while intact hCG levels have been shown to be either little altered (Cuckle *et al.*, 1988, Bogart *et al.*, 1990., Johnston *et al.*, 1991, Van Lith, 1992, Aitken *et al.*, 1993) or moderately elevated (Brock *et al.*, 1990, Kratzer *et al* 1991). Frß-hCG and PAPP-A appear to be the most useful markers for Down's syndrome, but Frß-hCG has the advantage that, unlike PAPP-A, it is a good predictor of Down's syndrome at all gestations between 7 and 20 weeks.

Little is currently known about the control of production of these various placental and fetal markers and how this might be altered in Down's syndrome and other chromosomally abnormal pregnancies. None of the feto-placental markers AFP, α or ß subunit of hCG, SP_1 or PAPP-A, has the coding gene located on chromosome 21. In second trimester Down's syndrome pregnancies hCG and Frß-hCG levels are considerably elevated, SP_1 is slightly elevated and PAPP-A levels unchanged. In the first trimester a different pattern is seen, with elevated levels of Frß-hCG, hCG levels which are little changed and reduced levels of SP_1 and PAPP-A.

It was originally suggested by Wald *et al.* (1988b) that the change in maternal serum marker levels in Down's syndrome pregnancies

might be due to immaturity of the feto-placental unit, leading to the pattern of AFP, hCG and UE3 levels more typical of those existing 2-3 weeks earlier in gestation. However the accumulation of more data on other pregnancy markers in both the first and second trimesters has confounded this simple explanation. For example the higher levels of SP_1 found in second trimester Down's syndrome pregnancies do not reflect the pattern of a few weeks earlier in gestation.

An understanding of the control of production and secretion of pregnancy markers by the feto-placental unit into the maternal circulation may contribute to improved design and performance of prenatal screening protocols.

6.9 CONCLUSIONS

Prenatal screening by biochemical methods described in retrospective studies and applied in routine clinical practice in a large unselected population have demonstrated that around two-thirds of Down's syndrome pregnancies can be detected in the second trimester. In addition to more than doubling the detection rate achievable using maternal age alone, the application of biochemical screening has been shown to be much more effective in practice, with increased utilization of diagnostic testing by women of all ages including women aged 35 years and over. This has resulted in a measurable decline in the birth incidence of Down's syndrome in the west of Scotland.

However, such screening is neither perfectly sensitive nor specific and the report of a positive screening result may generate significant anxiety in the patient. In addition the improvement in sensitivity has led to an increase in the number of women who undergo therapeutic termination of affected pregnancies at advanced gestations. Implementation of biochemical screening had thus created an increased counselling workload.

Prospects for improved sensitivity rest upon the discovery of new placental or fetal markers with greater predictive power than those currently in use. Improvements in specificity would reduce the size of the false positive group and thus reduce the burden of anxiety in the screened population and the number of women requiring counselling. Earlier detection of affected pregnancies by extension of screening to the first trimester and the consequent reduction in the trauma associated with late termination would be of major benefit to the patient.

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