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Review

Antenatal screening for Down's syndrome

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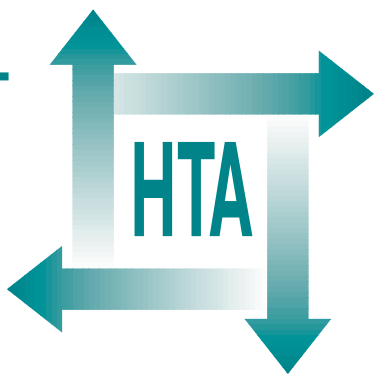
Health Technology Assessment
NHS R&D HTA Programme



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Antenatal screening for Down's syndrome

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This report is one of a series covering acute care, diagnostics and imaging, methodology, pharmaceuticals, population screening, and primary and community care. It was identified as a priority by the Population Screening Panel.

The views expressed in this publication are those of the authors and not necessarily those of the Standing Group, the Commissioning Board, the Panel members or the Department of Health. The editors wish to emphasise that funding and publication of this research by the NHS should not be taken as implicit support for the recommendations for policy contained herein. In England, policy options in this area are to be considered by the National Screening Committee, chaired by the Chief Medical Officer, who will take into account the views expressed here, further available evidence and other relevant considerations.

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Contents

Glossary and list of abbreviations

Technical terms and abbreviations are used throughout this report. The meaning is usually clear from the context but a glossary is provided for the non-specialist reader. In some cases usage differs in the literature but the term has a constant meaning throughout this review.

Glossary

Affected pregnancies Pregnancies in which the foetus has Down's syndrome.

Cut-off level The value of a screening variable which distinguishes screen positive from screen negative results.

Detection rate The proportion of affected pregnancies with screen positive results (also known as the sensitivity). This is independent of the prevalence of pregnancies with Down's syndrome.

False-negatives Affected pregnancies with screen negative results.

False-positives Unaffected pregnancies with screen positive results.

False-positive rate The proportion of unaffected pregnancies with screen positive results. When specified as the complement of the false-positive rate – that is, 100% – false-positive rate (%), it is called the specificity. This is independent of the prevalence of pregnancies with Down's syndrome.

Multiple of the median The serum marker concentration for a woman divided by the median concentration value for unaffected pregnancies of the same gestational age.

Odds of being affected given a positive result Self-defined – for example, an odds of being affected given a positive result of 1:20 means that among women with screen positive results there will, in expectation, be one affected pregnancy and 20 unaffected pregnancies. This is dependent on the detection and false-positive rates of the test and the prevalence of pregnancies with Down's syndrome. The odds of being affected given a positive result expressed as a proportion (in this example 1/21) is the positive predictive value of the screening test.

Risk This can be expressed in two ways:
(a) as an odds (for example, 1:3 – that is, one affected pregnancy for every three unaffected pregnancies)
(b) as a proportion (for example, the odds of 1:3 expressed as a proportion would be one-quarter – that is, one affected pregnancy out of a total of four pregnancies).

Screening The systematic application of a test or inquiry to identify individuals at sufficient risk of a specific disorder to benefit from further investigation or direct preventive action, among persons who have not sought medical attention on account of symptoms of that disorder.

Screen negative results A screening result that is less than the specified cut-off level. For example, if risk is the screening variable and the risk cut-off level is 1 in 250, a risk of 1 in 500 would be interpreted as screen negative.

Screen positive results A screening result that is greater than or equal to a specified cut-off level. For example, if risk is the screening variable and the risk cut-off level is 1 in 250, a risk of 1 in 200 would be interpreted as screen positive.

True-negatives Unaffected pregnancies with screen negative results.

True-positives Affected pregnancies with screen positive results.

continued

List of abbreviations

AFP	α-fetoprotein
Bart's	St Bartholomew's Hospital, London
CA 125	cancer antigen 125
CI	confidence interval
CVS	chorionic villus sampling
DAD	discriminant aneuploid detection
DR	detection rate*
DS	Down's syndrome*
EQAS	external quality assurance scheme
FACS	fluorescent activated cell sorting
FPR	false-positive rate*
hCG	human chorionic gonadotrophin
LR	likelihood ratio*
MACS	magnetic activated cell sorting
MCQ	multiple choice questionnaire*
MoM	multiple of median
NEQAS	National External Quality Assurance Scheme (UK)
NTD	neural tube defect
OAPR	odds of being affected given a positive result*
PAPP-A	pregnancy-associated plasma protein-A
SP1	schwangerschaftsprotein 1 (or pregnancy specific β_1 glycoprotein)
TA-CVS	transabdominal chorionic villus sampling*
TC-CVS	transcervical chorionic villus sampling*
uE_3	unconjugated oestriol
URNAP	urea-resistant neutrophil alkaline phosphatase

* Used only in figures and tables

Executive summary

Background

Over the past 15 years there have been notable advances in antenatal screening for Down's syndrome. First serum α-fetoprotein (AFP) and later human chorionic gonadotrophin (hCG) and unconjugated oestriol (uE_3), together with maternal age, have been widely used in screening for Down's syndrome, with a detection rate of about 70% for a 5% false-positive rate. More recently inhibin A has been added as a fourth serum marker.

Objectives

- To summarise the expected performance of serum and ultrasound makers for Down's syndrome.
- To evaluate the effectiveness, safety and cost-effectiveness of the different methods of antenatal screening and diagnosis.
- To review current screening practice for Down's syndrome in Britain.
- To specify the most appropriate method of Down's syndrome screening and identify areas for further research.

Methods

The literature on antenatal screening for Down's syndrome was reviewed.

Results

Principles of antenatal screening for Down's syndrome

Methods of screening need to be fully evaluated before being introduced into routine clinical practice. This includes choosing markers for which there is sufficient scientific evidence of efficacy, quantifying performance and establishing methods of monitoring performance. Screening services need to be well integrated and managed.

Serum markers at 15–22 weeks of pregnancy

Screening performance varies according to the choice of markers used and whether ultrasound is used to estimate gestational age. When the latter is used in combination with maternal age, the detection rate for a 5% false-positive rate is estimated to be 59% for the double test (AFP and hCG), 69% for the triple test (AFP, hCG, uE_3) and 76% for the quadruple test (AFP, hCG, uE_3, inhibin A).

Urinary markers and foetal cells in maternal blood

Urinary β-core hCG has been shown to be raised in Down's syndrome pregnancies. Urinary total oestriol and free β-hCG may also be of value but it would be premature to introduce them into screening practice.

Foetal cells can be identified in maternal circulation and techniques such as fluorescent *in situ* hybridisation can be used to identify Down's syndrome. However, this does not have the performance, simplicity or economy needed to replace existing methods.

Demonstration projects

Several demonstration projects using triple and double tests have been conducted, in which screening uptake was about 80% with screen positive rates of about 5–6%. Approximately 80% of women with positive results had an invasive diagnostic test, and about 90% of those found to have a pregnancy with Down's syndrome chose to have a termination.

Ultrasound markers at 15–22 weeks of pregnancy

There are a number of ultrasound markers of Down's syndrome at 15–22 weeks, of which nuchal fold thickness is the most discriminatory on its own, but not discriminatory enough for screening. The markers could be used in combination with the serum markers but no studies assessing this have been completed to date.

Serum and ultrasound screening at 10–14 weeks of pregnancy

The serum markers pregnancy-associated plasma protein-A (PAPP-A) and free β-hCG, combined with maternal age have an estimated detection rate of 62% for a 5% false-positive rate.

Nuchal translucency is a useful marker of Down's syndrome. There are differing estimates of screening performance and some are subject to bias. Further studies are needed to quantify the performance of this test alone and in combination with biochemical markers. There is also a need to compare the performance of such screening with screening at 15–22 weeks to determine which has the greater efficacy and which is the most cost-effective.

Methods of antenatal diagnosis

The standard method of antenatal diagnosis is amniocentesis at about 15 weeks of pregnancy followed by karyotyping of cultured cells from the amniotic fluid. The excess foetal loss attributed to amniocentesis is approximately 0.9%.

Before 15 weeks of pregnancy, transabdominal chorionic villus sampling (CVS), although less accurate than mid-trimester amniocentesis, seems to be the diagnostic method of choice.

Cost-effectiveness of serum screening

In general, serum screening is more cost-effective than screening based on maternal age alone at detection rates of about 50% or greater. As the number of screening markers increases, the cost per pregnancy screened increases but, if an extra marker is sufficiently discriminatory, the cost per Down's syndrome birth avoided may decline. For example, the estimated cost per pregnancy screened and the cost per Down's syndrome birth avoided is: £8.90 and £25,600 for the double test; £9.60 and £22,700 for the triple test, and £11.60 and £23,100 for the quadruple test.

Safety

Screening leads to women having an invasive diagnostic procedure that can result in foetal loss. As screening performance improves, the number of unaffected foetal losses per Down's syndrome birth avoided declines by 24%, from 0.59 (double test) to 0.45 (quadruple test).

Psychosocial aspects

Several studies have shown that the anxiety associated with screening is short lived and can be minimised by the provision of clear and simple information before screening, together with counselling for women with positive results.

Health professionals often do not have adequate knowledge of serum screening and therefore have difficulty in reporting screening results to women.

Quality assurance and monitoring

Quality assurance and monitoring should be an integral part of a screening service. It is currently not possible to tell whether screening centres undertake epidemiological monitoring and service audit satisfactorily.

Current screening practice

Serum screening for Down's syndrome has been widely introduced into practice and has enabled a substantially higher proportion of pregnancies to be identified without materially increasing the proportion of women requiring an invasive diagnostic procedure. Although the screening approach, using multiple markers concurrently, was novel, it has been introduced reasonably effectively using statistical methodology that has been accepted and empirically validated. There is also an active research programme being conducted alongside the clinical service. In spite of the achievements, a number of problems were identified – incomplete coverage of screening, inconsistent practice and a lack of overall direction. The introduction of alternative methods of screening has led to multiple stepwise screening in an uncoordinated manner which is confusing to staff and patients. Some research findings have been introduced into practice before being fully evaluated.

Conclusions

Implications for policy

The evidence indicates that screening using the triple test with maternal age is more effective, safe and cost-effective than the double test. The performance of the quadruple test including inhibin A appears somewhat better.

There is substantial variation in screening services for Down's syndrome throughout the UK. This needs to be rectified. The authors recommend that policy makers should ensure overall direction, with a written policy, specified funding and line responsibility, while preserving local commitment.

The authors suggest the establishment of local screening units (covering 15,000 births per year – about three to four maternity units) which would have full responsibility for their service. These would each have a dedicated screening coordinator who would work together with a screening consultant.

Inequity of access to the service and the current multiple, stepwise uncoordinated screening of Down's syndrome should be addressed. The tendency to offer more than one method of screening to the same women at different stages of pregnancy should be avoided.

There is evidence that better staff education and training is needed so that patients are adequately informed about screening and its implications.

Implications for research

Serum markers and nuchal translucency have been shown to be effective in screening for Down's syndrome in the first trimester. However, this needs further evaluation in carefully monitored pilot screening programmes before a decision is made to introduce first trimester screening into general routine practice.

Other research areas include the study of urinary markers and foetal cells in maternal blood.

Chapter 1
Background

The association between maternal age and risk of having a Down's syndrome pregnancy was published in 1933.[1] In 1959 the presence of an extra chromosome 21 was identified as the pathognomonic feature of Down's syndrome,[2] and this was followed in 1966 by the first report of a successful chromosome analysis of human amniotic fluid cells.[3] In 1968 the first antenatal diagnosis of Down's syndrome was made.[4] Screening on the basis of selecting women of advanced maternal age for diagnostic amniocentesis was gradually introduced into medical practice. The usual cut-off age was between 35 and 37 years, and an amniocentesis was usually carried out at about 16–18 weeks of pregnancy. Such screening, based on maternal age, identified about 30% of pregnancies with Down's syndrome by offering an amniocentesis to the oldest 5% of women.

In 1972 raised amniotic fluid α-fetoprotein (AFP) was shown to be associated with open neural tube defect (NTD) pregnancies.[5] Later, raised maternal serum levels of AFP were found to be associated with anencephaly.[6,7] With the first reports of raised serum AFP and open spina bifida,[8,9] antenatal screening became a possibility, and in 1977 the scientific basis for this was described.[10] This led to the first antenatal serum screening programme for birth defects.[10]

In 1983 a 28-year-old woman gave birth to a baby with trisomy 18 in the Albert Einstein College of Medicine in New York. During her pregnancy her AFP level had been tested as part of the NTD screening programme and had been found to be very low on two occasions. She asked her obstetrician, Dr Merkatz, if the low levels of AFP might have been connected with the chromosome abnormality. Her inquiry was not ignored by her obstetrician. He, together with his colleagues, assembled AFP data on 53 cases of pregnancies associated with various chromosomal abnormalities, including 25 cases of Down's syndrome. Forty-three of the 53 cases had serum AFP levels below the median value for unaffected pregnancies, and these results were presented at the Albany birth defects meeting in 1983 and published in 1984.[11]

The findings of Merkatz and colleagues prompted Cuckle and colleagues in Britain to review data on serum AFP in Oxford, restricting their inquiry to Down's syndrome. AFP levels in 61 pregnancies with Down's syndrome were compared with those of 36,652 unaffected pregnancies between 14 and 22 weeks of pregnancy, and the median value for affected pregnancies was found to be about 25% lower than for unaffected pregnancies.[12] This association was found to be independent of maternal age and so information on both maternal age and serum AFP level could be combined to assess the risk of a Down's syndrome pregnancy; women above a specified risk cut-off level were considered screen positive and offered a diagnostic amniocentesis. This new method of screening identified about 35% of pregnancies with Down's syndrome, while maintaining a 5% false-positive rate (the proportion of unaffected pregnancies with screen positive results),[13] a 5% greater detection rate than with maternal age alone.

In 1987 levels of maternal serum human chorionic gonadotrophin (hCG) were shown to be about twice as high in Down's syndrome pregnancies as in unaffected pregnancies.[14] Reports followed showing that levels of maternal serum unconjugated oestriol (uE_3) were about 25% lower in Down's syndrome pregnancies than in unaffected pregnancies.[15,16] These two serum markers, together with AFP and maternal age, then formed the basis of the 'triple test' (sometimes known in Britain as the Bart's test). Performed between 15 and 22 weeks of pregnancy, it could identify about 60% of Down's syndrome pregnancies while maintaining a false-positive rate of about 5%.[17] The test showed the value of so-called risk screening, in which the risk estimate is itself used as the screening variable. It enables maternal age and three blood measurements to be interpreted together in a way that maximises the detection rate for a given false-positive rate. It is the most efficient method of screening based on the information available and the most equitable, ensuring that women with the highest risk are offered an amniocentesis.

Screening for Down's syndrome using various combinations of the serum markers was gradually introduced into the UK and the USA, and in 1992 the first demonstration projects on using the triple test in routine practice were published.[18,19] Other serum markers between 15 and 22 weeks of

pregnancy were subsequently identified, including the free subunits of hCG (α and β). In 1994 it was shown that serum screening at this time in pregnancy using AFP, uE_3, free β-hCG, and free α-hCG, and with gestational age routinely estimated by an ultrasound scan, could identify over 70% of Down's syndrome pregnancies for a 5% false-positive rate.[20] In 1996 dimeric inhibin A was shown to increase the detection rate to 76% for the same false-positive rate when used in combination with AFP, uE_3, and total hCG.[21–23]

Surveys of the extent of Down's syndrome screening in Britain were carried out in 1992[24] and 1994.[25] Information on screening practice was obtained from all health districts and boards in Britain. The proportion of health districts and boards offering multiple marker serum screening (AFP and at least one other serum marker) to all pregnant women increased from 25% in 1991 to 56% in 1994. About a quarter of health districts and boards still used screening based on maternal age alone and some (16%) restricted serum screening to women above a certain age.

In 1991 it was shown that low levels of a protein called pregnancy-associated plasma protein A (PAPP-A) were associated with Down's syndrome pregnancies before 15 weeks of pregnancy,[26] as were raised levels of free β-hCG.[27] If these two serum markers are combined with maternal age, 62% of Down's syndrome pregnancies can be identified with a false-positive rate of 5%.[28]

Ultrasound screening for Down's syndrome is being introduced into some centres in the UK and abroad. The most significant marker is that of a widened space at the back of the foetal neck, between the spine and the skin, in Down's syndrome pregnancies. This was first reported in second trimester foetuses in 1985,[29] and called an increased nuchal fold. In 1990 a similar finding called 'increased nuchal translucency', thought to be due to excess nuchal fluid accumulation in Down's syndrome foetuses, was reported at about 10–11 weeks of pregnancy.[30] In 1992 a study showed the potential of this measurement as a screening test for Down's syndrome at this time in pregnancy.[31]

In this review of the literature on antenatal screening for Down's syndrome we estimate the expected performance of the serum and ultrasound markers. The results of the demonstration projects that describe their introduction into routine antenatal care are examined. We focus on the quantitative evaluation of efficacy, safety, and cost-effectiveness of the different methods of antenatal screening and diagnosis. Studies on the psychosocial aspects of screening are reviewed with the aims of specifying the most appropriate method of Down's syndrome screening and identifying those areas in which further research is needed.

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Chapter 2

Principles of antenatal screening for Down's syndrome

Introduction

Antenatal screening and selective termination offer the possibility of preventing the birth of infants with serious congenital abnormalities. Screening is not without costs, both human and financial. Screening will miss some affected pregnancies (false-negative results) and will identify some unaffected pregnancies as screen positive (false-positive results). The performance of different methods of screening therefore needs to be quantified, and the method of choice would be the one that maximises the detection rate for a given false-positive rate. The consequences of offering an antenatal screening programme also need to be considered, so that systems can be implemented to facilitate an effective and compassionate service.

A special consideration in Down's syndrome screening is the need to combine the values of several screening markers simultaneously (for example, maternal age, AFP, and hCG). It is inefficient to consider these tests individually, or to offer them sequentially, because this will mean that more women will have an invasive diagnostic procedure to identify the same number of affected pregnancies. The markers have different units of measurement (years and multiples of median (MoMs)) and the ability to predict Down's syndrome is not the same for a unit of AFP as for a unit of hCG. A common currency is needed, and the only practical way to combine several markers concurrently is to convert each measurement into a combined risk estimate. The risk estimate then becomes the screening variable. The risk of having a Down's syndrome pregnancy can be estimated for each woman based on her age and any combination of serum markers, and she is designated screen positive if her risk value exceeds a specified cut-off (say, 1 in 250).

Ethical aspects

Special ethical considerations apply to screening. In ordinary clinical practice, investigations are carried out on individuals with symptoms who seek medical help; the clinician's obligation is to treat the patient in the best way possible, even if there is incomplete knowledge about the disease or its remedy. It may not be possible to predict reliably the outcome of the action taken. In screening, the position is somewhat different; healthy individuals are approached for investigation and a small percentage are identified as being at sufficiently high risk of a disorder to justify an intervention that, because of its cost or risk of hazard, is not offered to everyone. There is, therefore, an obligation not to initiate any action unless the consequences of doing so are quantified and predictable and, of course, there is an effective remedy available.

Basic requirements for a worthwhile screening programme

There is much interest in new methods of screening for Down's syndrome and, in many cases, such methods have been introduced into routine clinical practice before being fully evaluated. *Table 1* shows the basic requirements for a worthwhile screening programme that should be fulfilled by any screening procedure (taken from Cuckle and Wald, 1984[1] with the addition of one requirement: 'access').

Risk screening

Risk screening depends on using a statistical model[2] because it is impractical to collect a sufficiently large database to determine empirically the risks of Down's syndrome for every combination of values of the different serum markers used. In general, univariate and multivariate Gaussian models, which have been empirically validated, have been used in serum screening for Down's syndrome.[3]

Any statistical model has limitations. Data in the tails of the distribution are likely to fit theoretical models relatively poorly. It is important in risk screening that the statistical model used is explicitly specified, the data used to construct the model are published, and that the limits over which the model is judged to be satisfactory are defined. Statistically, the risk model will be specified by the means and

TABLE 1 *Basic requirements for a worthwhile screening programme*

Aspect	Requirement
Disorder	Well defined
Prevalence	Known
Natural history	Medically important disorder for which there is an effective remedy available
Financial	Cost-effective
Facilities	Available or easily installed
Ethical	Procedures after a screen positive result are generally agreed and acceptable both to the screening authorities and to the patients
Test	Simple and safe
Test performance	Distributions of test values in affected and unaffected individuals known, extent of overlap sufficiently small, and a suitable cut-off level defined
Access	All people who may benefit from a screening test should have access to it

standard deviations of the individual markers in affected and unaffected pregnancies and by the correlation coefficients between all combinations.

Estimates of these statistical parameters for all the markers used are best obtained by measuring them all in the same data set of affected and unaffected pregnancies. This condition is satisfied by the sample of 77 Down's syndrome pregnancies and 385 controls collected in Oxford.[2] This was supplemented by a set from St Bartholomew's Hospital (Bart's) on about 2000 unaffected pregnancies. These have ultrasound dating information which permits the derivation of statistical parameters relating to gestational age based on an ultrasound scan examination, as well as gestation estimated from a woman's last menstrual period. Other data sets have examined individual markers and several markers in combination, but none covers the full range of markers in the same data set and none provides separate sets of parameters relating to the two alternative methods of estimating gestational age. Analyses in this report are, therefore, principally based on the Oxford– Bart's data set, but we use the results of meta-analyses of published studies for corroboration and use the Oxford–Bart's results only if they are consistent with other evidence.

Several factors affect serum marker concentrations (for example, gestational age, maternal weight, ethnic group), and some of these should be accounted for by using appropriate regression techniques. It is not appropriate to incorporate all the factors into a single multiple regression equation as all the items may not be available for every pregnancy. It is preferable to allow for each factor separately, with the primary regression being the serum marker on gestational age alone to determine normal median values. The gestational age regression is thereby unaffected by other variables and the screener can examine the effect of the different adjustment factors separately. This method has been the most widely adopted.

Determining normal median values of the serum markers is a first step in risk estimation. Strictly this should be based on unaffected pregnancies but, because Down's syndrome is relatively rare, their values will have a negligible effect on the normal median, which can therefore be accurately estimated from values on all screened women. The concentration of each marker assayed for each woman is then divided by the normal median for women of the same gestational age to convert the concentration into a multiple of the normal median (its MoM value). If the estimates of the medians are found to be inaccurate – for example, owing to assay drift – they need to be corrected. The process requires monitoring and judgement; it should not be carried out automatically by computer.

Estimating detection and false-positive rates

The screening test for Down's syndrome is used to classify women as having either positive or negative results on the basis of their estimated risk, calculated from a combination of their age and serum marker levels. The performance of the screening test is measured by both the detection rate (the proportion of affected pregnancies with positive results) and the false-positive rate (the proportion of unaffected pregnancies with positive results).

Ideally, an estimation of screening performance would be made directly in a large population of affected and unaffected pregnancies. However, because collecting the required numbers to do this is impractical, a Gaussian model is used. The statistical parameters of the Gaussian distribution for a combination of serum markers (means, standard deviations and correlations obtained from a sufficient sample of affected and unaffected pregnancies), together with the age distribution of pregnancies in a particular population and the age-specific risk, are used to generate a hypo-

thetical large population of women with affected and unaffected pregnancies. A risk estimate is then calculated for each woman. The number of affected pregnancies that exceeds a specified risk cut-off is counted and expressed as a proportion of all affected pregnancies to give the detection rate. Similarly, the number of unaffected pregnancies which exceeds the cut-off is expressed as a proportion of all unaffected pregnancies to give the false-positive rate. The procedure is performed with the aid of a computer and has been described in detail elsewhere.[4,5] From this, we can obtain the distribution of risk estimates in affected and unaffected pregnancies, which can be represented as overlapping distribution curves *(Figure 1)*.

The multivariate Gaussian model is useful in that it is modular. It only depends on the statistical parameters (means, standard deviations, and correlations), and these can be easily compared with those obtained by other researchers. If the distribution of the markers in the population is Gaussian, then the model will be robust and the estimate of screening performance will be accurate. The model is less influenced by outliers and sample size. It is the most commonly used method of estimating screening performance.

Other published modelling methods to estimate screening performance include logistic regression,[5] discriminant function analysis (discriminant aneuploid detection (DAD)[7]) and the ratio method described by Crossley and colleagues.[8] The first two, logistic regression and DAD, are more data-derived than the Gaussian model and tend to put too much weight on peculiarities in the data used. The logistic regression model may be better than the Gaussian model if the distributions of the markers are non-Gaussian. The DAD model has been shown to be less effective than the Gaussian model.[9,10] The ratio method can only be used for two markers and does not take into account the difference in the relative importance between the two markers.

Organisational requirements

In setting up a screening programme it is necessary to adopt a method of screening and choose markers for which there is sufficient scientific evidence of efficacy. The screening policy must be specified and quantified so that its overall effect among the women screened is predictable and can be monitored. There has to be a clear division into two groups of women who are screened: those women at high enough risk to justify the offer of amniocentesis or alternative invasive diagnostic procedure (screen positive) and those not considered sufficiently at risk (screen negative). Women who are screen positive can be given the actual risk estimate when deciding whether to accept diagnostic testing. Although the estimate is not usually reported to screen negative women, it should be available on request. Failure to classify women as screen positive or screen negative will mean that there is no screening policy. This can preclude effective quality control and monitoring, and may

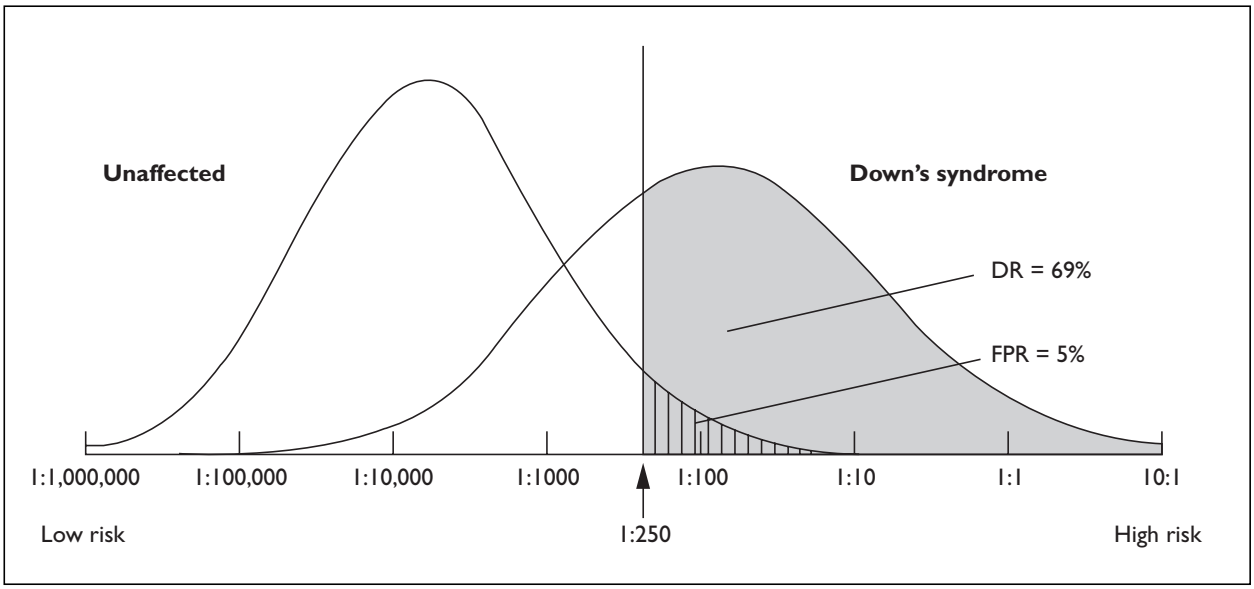

FIGURE 1 *Distribution of the risk of having a Down's syndrome pregnancy in affected and unaffected pregnancies using maternal age with AFP, uE_3 and hCG (gestational age estimated by scan and marker levels are adjusted for maternal weight)[6] (DR, detection rate; FPR, false-positive rate)*

lead to confusion and lack of confidence in the screening programme. It could encourage programmes that are not as effective or safe as they should be, and may not be financially cost-effective.

Screening needs to be provided as an integrated service. The screening programme entails many separate episodes which need to be coordinated and managed. These include the assay of the markers, their biochemical interpretation to obtain an accurate risk estimate, the use of ultrasound to date the pregnancy, counselling and carrying out antenatal diagnosis soon after the woman has been classified screen positive. Overall, screening must not be a piecemeal opportunistic series of clinical interventions, but a well-organised, predictable process that people consent to join with knowledge and understanding. A team approach is needed, with a screening consultant having the responsibility to adjust screening policy and method of delivery of the service as appropriate, taking into account the availability of resources and views of colleagues from relevant disciplines. Unfortunately, in practice this is often not the case; decision-making is often divided without a coherent screening strategy.

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Chapter 3

Serum markers at 15–22 weeks of pregnancy

Serum markers

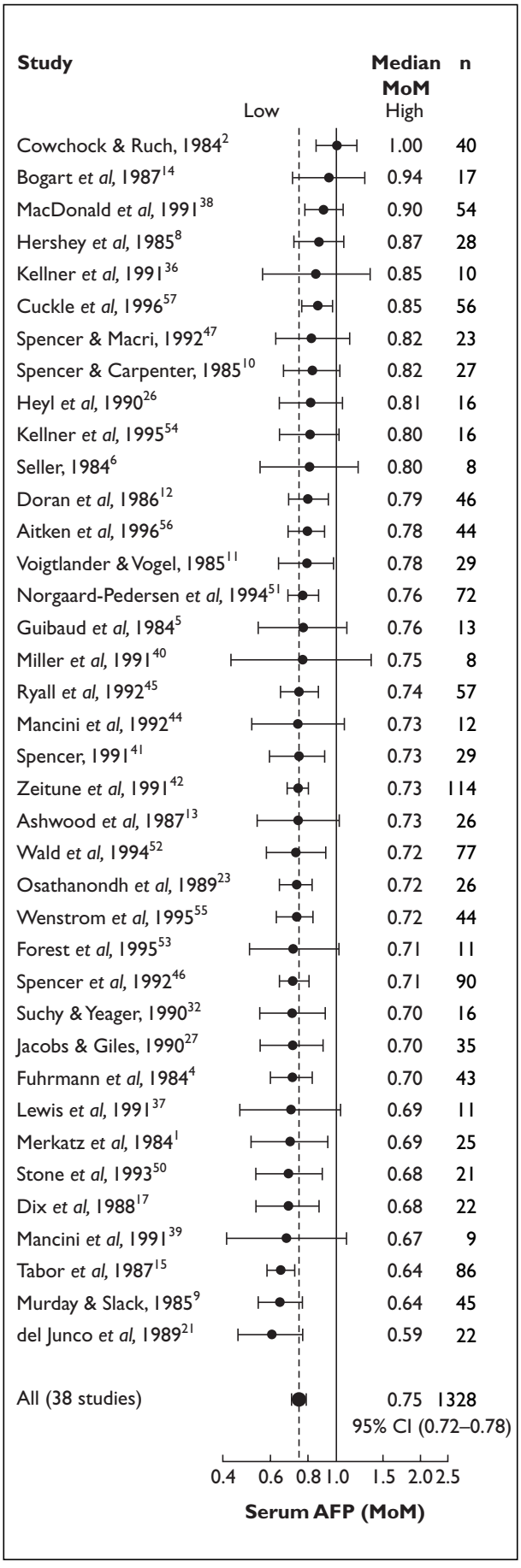
The serum markers that have been found to have been of value for Down's syndrome screening between 15 and 22 weeks of pregnancy are AFP, uE_3, total hCG, free β-hCG, free α-hCG, and dimeric inhibin A.[1–63] In addition to these markers, cancer antigen 125 (CA 125), schwangerschafts-protein 1 (also known as pregnancy specific β_1 glycoprotein) (SP1), PAPP-A, the α subunit of inhibin (α inhibin), and urea-resistant neutrophil alkaline phosphatase (URNAP) have been investigated *(Table 2)*, but two (CA 125 and PAPP-A) were not found to be useful, SP1 and α inhibin had little value when added to established markers, and URNAP is still under research. *Figure 2* shows the median (or mean) and 95% confidence interval (CI) for AFP in affected pregnancies expressed in MoMs for unaffected pregnancies at 15–22 weeks of pregnancy. *Figures 3* to *7* show, in a similar way, the estimates for other serum markers. The figures show the individual point estimates from each published study, together with the number of Down's syndrome pregnancies and the 95% CI around the point estimate. A summary estimate, combining information from all the studies using the method described by DerSimonian and Laird,[82] is also given. *Figure 8* shows each of the individual point estimates, together with the point estimate derived from the Oxford–Bart's data set,[52,60,61] which are used in this report to estimate screening performance for various combinations of serum markers. The two sets of estimates are in close agreement.

Table 3 compares all the statistical parameters (means, standard deviations, and correlation coefficients) for AFP, uE_3, hCG and its subunits (free α- and β-hCG) from the three research groups with the largest number of Down's syndrome pregnancies, that each assessed the distributions of having at least three markers in the same data set.[45,46,52] The estimates are reasonably similar.

It has been suggested that the risk for Down's syndrome should be calculated using statistical parameters based on combining data from different data sets by means of a meta-analysis rather than by using estimates from a single study.[83] The advantage of this approach is that by using several data sets a larger number of cases are available and the parameters can be estimated more precisely. The disadvantage, which we believe is more important, is its complexity and the possibility that there will be statistical inconsistencies in the estimates of the parameters in the multivariate model, which can result in an insoluble calculation of risk.[84,85]

TABLE 2 *Down's syndrome pregnancies at 15–22 weeks of pregnancy: median MoM (and 95% CI) for CA 125, SP1, α inhibin, PAPP-A and URNAP*

Serum marker and study	Down's syndrome (n)	Median MoM	95% CI
CA 125			
Spencer, 1991[64]	25	0.93	(0.72–1.20)
Hogdall *et al*, 1992[65]	15	0.63	(0.12–3.41)
van Blerk *et al*, 1992[66]	10	0.72	(0.47–1.11)
van Lith *et al*, 1993[67]	9	0.90	(0.56–1.45)
Wenstrom *et al*, 1997[63]	22	1.29	(1.05–1.58)
All (5 studies)	**81**	**0.94**	**(0.74–1.21)**
SP1			
Bartels & Lindemann, 1988[68]	24	2.10	(1.64–2.68)
Knight *et al*, 1989[69]	24	1.53	(1.18–1.98)
Wald *et al*, 1989[70]	77	1.20	(1.05–1.37)
Bartels *et al*, 1990[25]	43	1.28	(1.03–1.59)
Petrocik *et al*, 1990[71]	46	2.08	(1.75–2.48)
Graham *et al*, 1992[72]	48	1.17	(1.01–1.35)
Qin *et al*, 1997[73]	117	1.28	(1.11–1.49)
All (7 studies)	**379**	**1.47**	**(1.23–1.76)**
α Inhibin			
van Lith *et al*, 1992[74]	10	1.90	(1.28–2.83)
Spencer *et al*, 1993[75]	15	3.65	(1.78–7.47)
Cuckle *et al*, 1994[76]	19	1.31	(0.92–1.87)
Lambert-Messerlian *et al*, 1996[58]	20	0.81	(0.43–1.53)
All (4 studies)	**64**	**1.63**	**(1.01–2.62)**
PAPP-A			
Cuckle *et al*, 1992[77]	18	0.87	(0.62–1.23)
Wald & Voller, 1992[78]	16	1.02	(0.54–1.92)
Knight *et al*, 1993[79]	30	1.01	(0.81–1.26)
All (3 studies)	**64**	**0.97**	**(0.84–1.11)**
URNAP			
Grozdea *et al*, 1983[80]	4	1.84	(0.09–39.39)
Cuckle *et al*, 1990[81]	72	1.65	(1.56–1.74)
All (2 studies)	**76**	**1.65**	**(1.57–1.74)**



We used the Oxford-Bart's data set as the basis of most of our statistical parameters, because it is the only one that has all the current markers of interest, including inhibin A, with estimates according to whether gestational age was estimated by dates or ultrasound scan.

LEFT: FIGURE 2 *Median AFP (MoM) at 15–22 weeks in Down's syndrome pregnancies, with 95% CI and the number of affected pregnancies in each study (the mean value was used when the median value could not be obtained). Studies are ranked according to the median MoM. The pooled median is indicated by the vertical broken line*

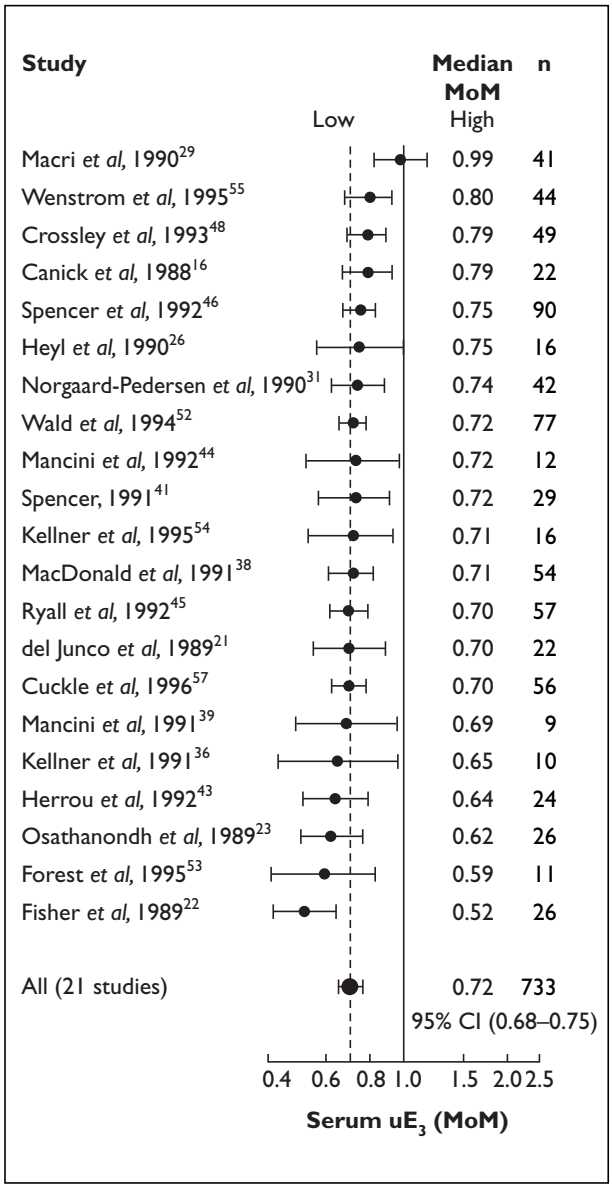


FIGURE 3 *Median uE_3 (MoM) at 15–22 weeks in Down's syndrome pregnancies, with 95% CI and the number of affected pregnancies in each study (the mean value was used when the median value could not be obtained). Studies are ranked according to the median MoM. The pooled median is indicated by the vertical broken line*

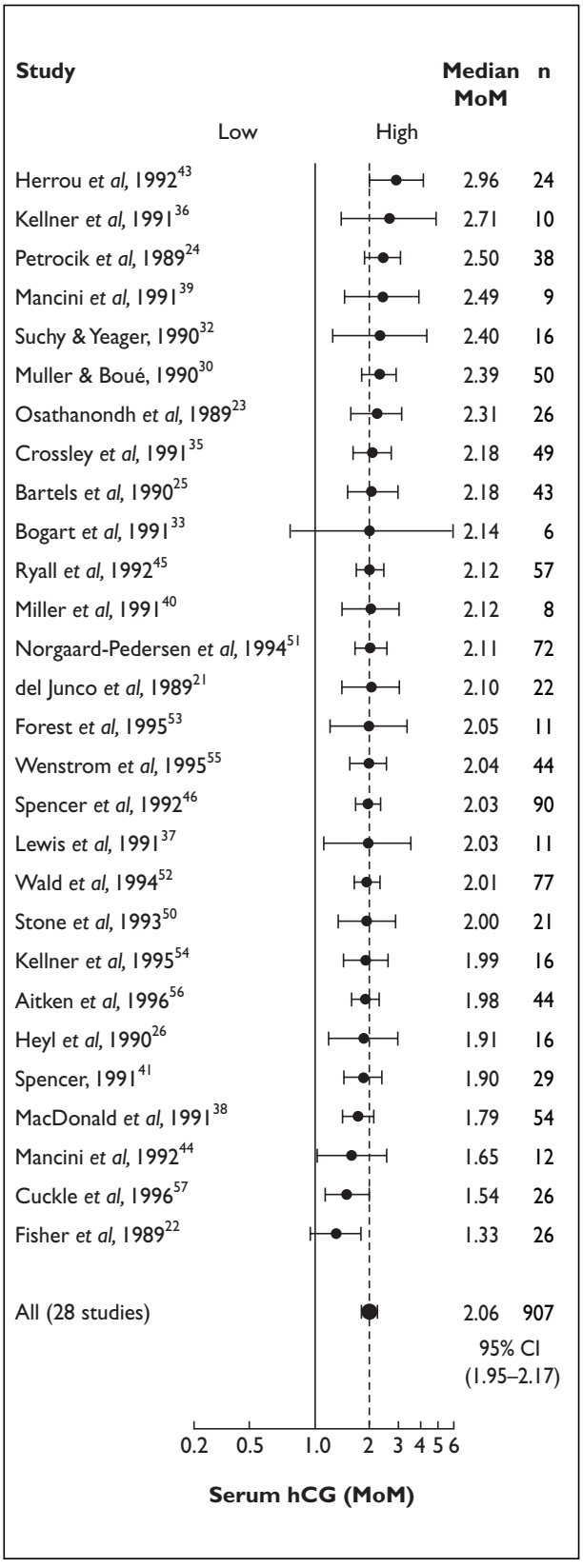


FIGURE 4 *Median hCG (MoM) at 15–22 weeks in Down's syndrome pregnancies, with 95% CI and the number of affected pregnancies in each study (the mean value was used when the median value could not be obtained). Studies are ranked according to the median MoM. The pooled median is indicated by the vertical broken line*

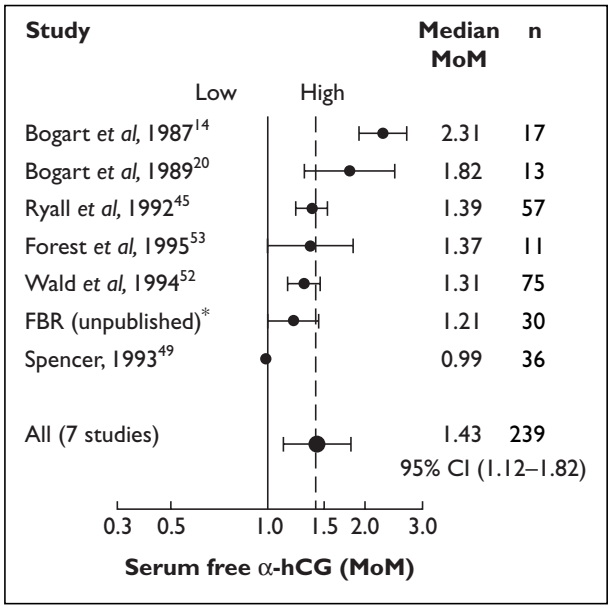


FIGURE 5 *Median free α-hCG (MoM) at 15–22 weeks in Down's syndrome pregnancies, with 95% CI and the number of affected pregnancies in each study (the mean value was used when the median value could not be obtained). Studies are ranked according to the median MoM. The pooled median is indicated by the vertical broken line*

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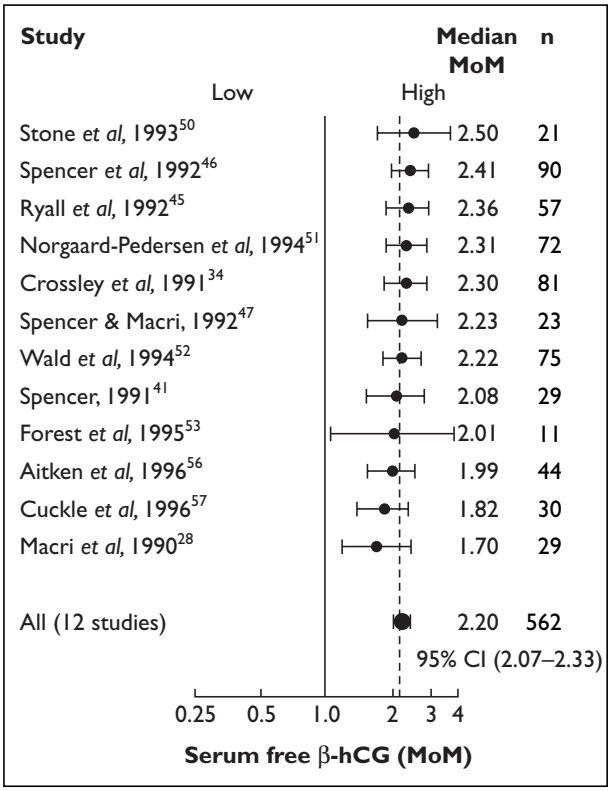

FIGURE 6 *Median free β-hCG (MoM) at 15–22 weeks in Down's syndrome pregnancies, with 95% CI and the number of affected pregnancies in each study (the mean value was used when the median value could not be obtained). Studies are ranked according to the median MoM. The pooled median is indicated by the vertical broken line*

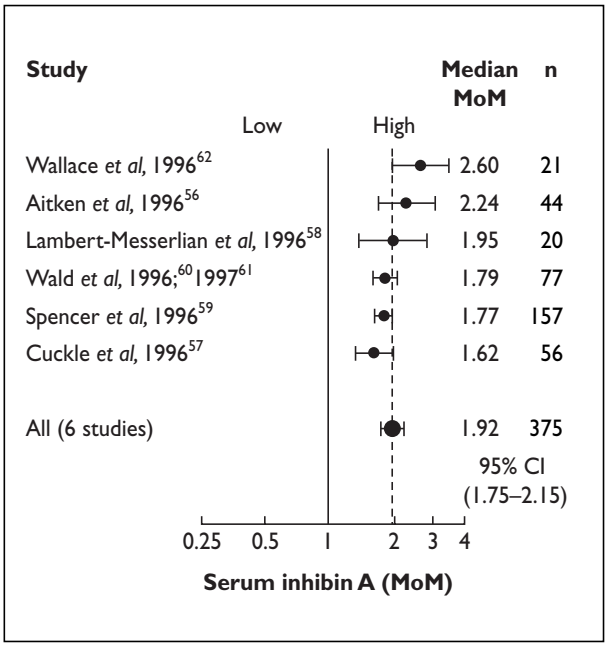

FIGURE 7 *Median dimeric inhibin A (MoM) at 15–22 weeks in Down's syndrome pregnancies, with 95% CI and the number of affected pregnancies in each study (the mean value was used when the median value could not be obtained). Studies are ranked according to the median MoM. The pooled median is indicated by the vertical broken line*

Screening performance

Table 4 shows the detection rate for a 5% false-positive rate and the odds of being affected given a positive result for all combinations of maternal age and serum markers, after allowing for the effect of maternal weight on serum marker levels.[52,60,61] Screening performance is shown according to the method of dating gestational age – namely, based on dates (time since the first day of the last menstrual period) or ultrasound scan (using crown rump length or biparietal diameter). Total hCG and its free β subunit are the most discriminatory markers when used on their own with maternal age, both giving a detection rate of 49% for a 5% false-positive rate.

Most serum screening programmes use multiple markers – namely, the double test (maternal age with AFP and either total hCG or free β-hCG) or the triple test (maternal age with AFP, uE_3, and total hCG). *Figure 9* shows the detection rate for a 5% false-positive rate using maternal age and multiple marker combinations.There is a considerable increase in the detection rate when multiple markers are used. About twice the number of

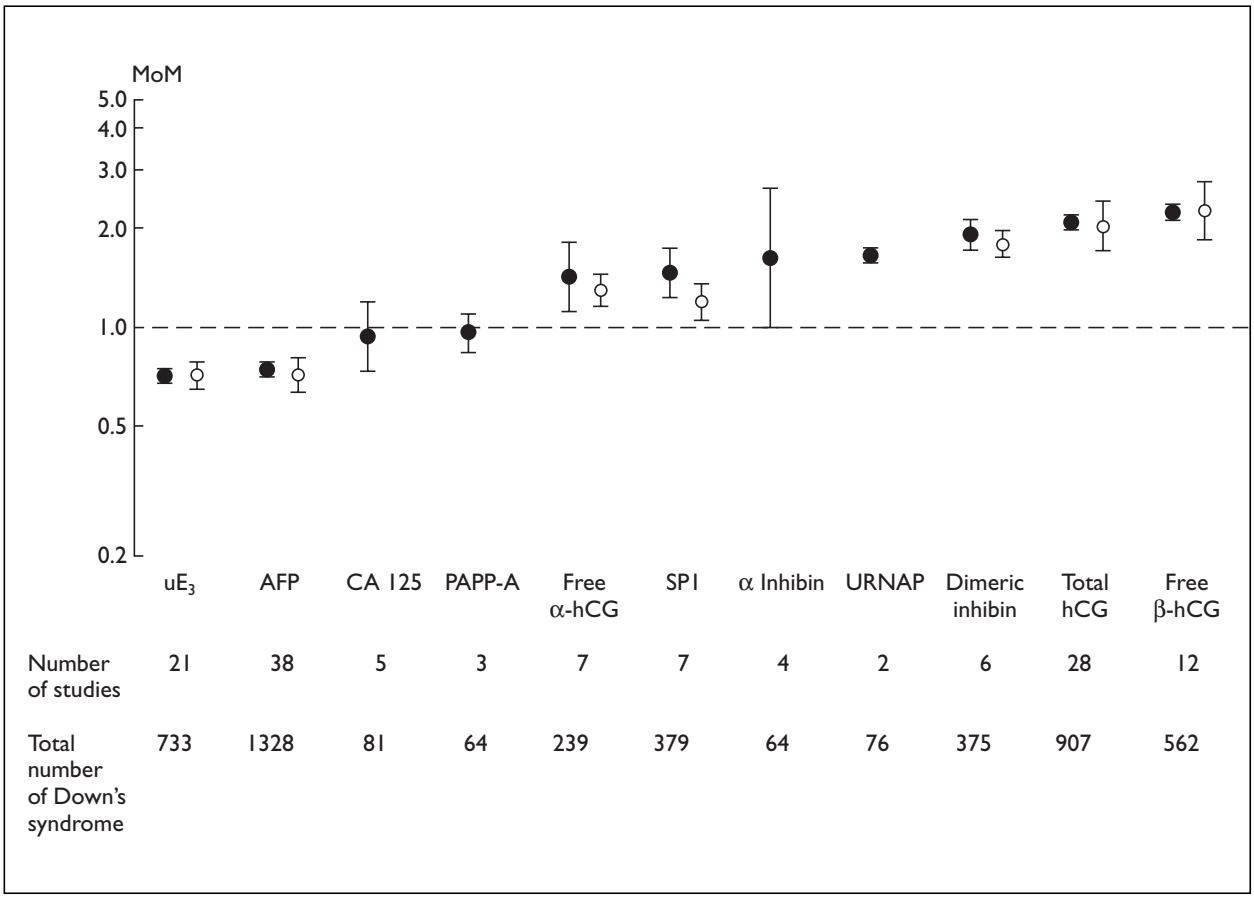

FIGURE 8 *The pooled median and 95% CI (solid circle) at 15–22 weeks from the studies shown in* Figures 2 *to 7. The open circles indicates the estimate from the Oxford–Bart's data set*[52,60,61]

TABLE 3 *Published statistical parameters (medians, standard deviations, and correlation coefficients) from three groups*

Serum marker	Wald *et al*, 1994[52]		Spencer *et al*, 1992[46]		Ryall *et al*, 1992[45]	
	Down's syndrome (n = 77)*	Unaffected (n = 385)*	Down's syndrome (n = 90)	Unaffected (n = 2862)	Down's syndrome (n = 57)	Unaffected (n = 171)
Median (antilog of log_{10} mean)						
AFP	0.72	1.00	0.71	1.00	0.74	1.00
uE_3	0.72	1.00	0.75	1.00	0.70	1.00
hCG	2.01	1.00	2.03	1.00	2.12	1.00
Free α-hCG	1.31	1.00	–	–	1.39	1.00
Standard deviation (log_{10})						
AFP	0.2015	0.1986	0.2013	0.1931	0.2015	0.1542
uE_3	0.1478	0.1391	0.2101	0.1476	0.1785	0.1472
hCG	0.2665	0.2401	0.2825	0.2410	0.2462	0.2879
Free α-hCG	0.1772	0.1520	–	–	0.1524	0.1637
Free β-hCG	0.3067	0.2508	0.3316	0.2544	0.3010	0.2945
Correlation (log_{10})						
AFP, uE_3	0.3359	0.2853	0.3740	0.3050	0.4780	0.3170
AFP, hCG	0.1681	0.0327	–0.1180	0.1520	0.0490	0.1640
AFP, free α-hCG	0.0824	0.1401	–	–	0.2570	0.4140
AFP, free β-hCG	0.1499	0.0125	0.1840	0.0190	–0.0230	0.0400
uE_3, hCG	–0.3565	–0.1423	–0.2930	–0.1190	–0.2200	–0.2160
uE_3, free α-hCG	0.0948	0.2273	–	–	0.0700	0.2400
uE_3, free β-hCG	–0.4486	–0.1770	0.0520	0.0270	–0.2210	–0.2320
hCG, free α-hCG	0.4599	0.3235	–	–	0.4210	0.3930
hCG, free β-hCG	0.8898	0.8838	0.8050	0.8170	0.9070	0.6820
Free α-hCG, free β-hCG	0.2162	0.1539	–	–	0.1690	0.1170

* *The standard deviations and correlations were derived using two data sets: (a) 77 Down's syndrome pregnancies and 385 matched unaffected controls; (b) 970 unaffected pregnancies.*

As gestational age was largely estimated by dates and not corrected for maternal weight in Spencer et al, *1992[46] and Ryall* et al, *1992,[45] the equivalent parameters in Wald* et al, *1994[52] are shown.*

For each marker, the median in unaffected pregnancies has been adjusted to 1.00, and the same adjustment applied to Down's syndrome pregnancies.

Down's syndrome pregnancies can be detected using the double test compared with screening using maternal age alone. The addition of uE_3 to AFP and total hCG increases the detection rate by about 5% if gestational age is estimated by dates, or by about 10% if it is based on an ultrasound scan. The quadruple test (the triple test plus inhibin A) has a detection rate of 76% if gestational age is based on an ultrasound scan.

Table 6 shows the false-positive rates for specified detection rates for different combinations of markers, and *Table 7* shows the detection rates for specified false-positive rates. At high detection rates the differences in performance are best observed by fixing the detection rate and comparing false-positive rates.

All the combinations shown in the figures and tables are part of established screening programmes, except the combination with inhibin A, which has only been recently introduced into routine screening practice. At present, the best combination of markers is the quadruple test with inhibin A, which can detect about three out of every four pregnancies with Down's syndrome but without offering an amniocentesis to more women than current screening programmes (5%).

Tables 8, 9 and *10* show the detection and false-positive rates according to risk cut-off level and method of estimating gestational age. *Table 8* relates to using one or two serum markers, *Table 9* to three markers, and *Table 10* to four markers. All combinations are given.

TABLE 4 *Screening performance for all combinations of serum markers[*] (all results have been corrected for maternal weight)*

Marker(s)	Gestational age estimated by			
	Dates		Scan	
	DR (%) for a 5% FPR	OAPR[†]	DR (%) for a 5% FPR	OAPR[†]
Maternal age alone (≥ 36 years)	30	1:130	30	1:130
Maternal age with one marker:				
AFP	36	1:110	37	1:105
uE_3	41	1:95	49	1:80
Total hCG	49	1:80	51	1:75
Free α-hCG	38	1:100	39	1:100
Free β-hCG	49	1:80	51	1:75
Inhibin A	44	1:90	44	1:90
Maternal age with two markers:				
AFP, uE_3	45	1:85	54	1:70
AFP, hCG	54	1:70	59	1:65
AFP, free α-hCG	45	1:85	47	1:80
AFP, free β-hCG	54	1:70	58	1:65
AFP, inhibin A	53	1:75	54	1:70
uE_3, hCG	56	1:70	64	1:60
uE_3, free α-hCG	53	1:70	60	1:65
uE_3, free β-hCG	57	1:70	64	1:60
uE_3, inhibin A	57	1:70	63	1:60
hCG, free α-hCG	51	1:75	53	1:75
hCG, inhibin A	58	1:70	59	1:65
Free α-hCG, free β-hCG	55	1:70	55	1:70
Free α-hCG, inhibin A	51	1:75	51	1:75
Free β-hCG, inhibin A	58	1:65	59	1:65
Maternal age with three markers:				
AFP, uE_3, hCG	59	1:65	69	1:55
AFP, uE_3, free α-hCG	56	1:70	64	1:60
AFP, uE_3, free β-hCG	60	1:65	68	1:55
AFP, uE_3, inhibin A	60	1:65	67	1:57
AFP, hCG, free α-hCG	57	1:65	60	1:65
AFP, hCG, inhibin A	64	1:60	68	1:55
AFP, free α-hCG, inhibin A	58	1:65	60	1:65
AFP, free β-hCG, inhibin A	64	1:60	67	1:55
AFP, free α-hCG, free β-hCG	60	1:65	62	1:60
uE_3, hCG, free α-hCG	60	1:65	67	1:60
uE_3, hCG, inhibin A	64	1:60	71	1:55
uE_3, free α-hCG, inhibin A	63	1:60	69	1:55
uE_3, free β-hCG, inhibin A	64	1:60	70	1:55
uE_3, free α-hCG, free β-hCG	62	1:60	69	1:55
Inhibin A, hCG, free α-hCG	59	1:65	60	1:65
Free α-hCG, free β-hCG, inhibin A	61	1:65	62	1:60

[*] *Excluding combinations that include both hCG and free β-hCG.*

[†] *Odds of being affected given a positive result (OAPR), rounded to nearest 5.*

Commonly used marker combinations are indicated in bold.

Source: Screening performance estimated using statistical parameters in Wald et al, *1994;[52] 1996;[60] 1997.[61]*

DR = detection rate; FPR = false-positive rate.

continued

TABLE 4 contd *Screening performance for all combinations of serum markers[*] (all results have been corrected for maternal weight)*

Marker(s)	Gestational age estimated by			
	Dates		Scan	
	DR (%) for a 5% FPR	OAPR[†]	DR (%) for a 5% FPR	OAPR[†]
Maternal age with four markers:				
AFP, uE_3, hCG, inhibin A	67	1:55	76	1:50
AFP, uE_3, free α-hCG, inhibin A	66	1:60	73	1:55
AFP, uE_3, free β-hCG, inhibin A	67	1:55	75	1:50
AFP uE_3, hCG, free α-hCG	63	1:60	72	1:55
AFP, uE_3, free α-hCG, free β-hCG	65	1:60	73	1:55
AFP, inhibin A, hCG, free α-hCG	65	1:60	69	1:55
AFP, free α-hCG, free β-hCG, inhibin A	67	1:60	69	1:55
uE_3, inhibin A, free α-hCG, hCG	66	1:60	73	1:55
uE_3, free α-hCG, free β-hCG, inhibin A	68	1:58	73	1:55

[*] *Excluding combinations that include both hCG and free β-hCG.*

[†] *Odds of being affected given a positive result (OAPR), rounded to nearest 5.*

Commonly used marker combinations are indicated in bold.

Source: Screening performance estimated using statistical parameters in Wald et al, *1994;[52] 1996;[60] 1997.[61]*

DR = detection rate; FPR = false-positive rate.

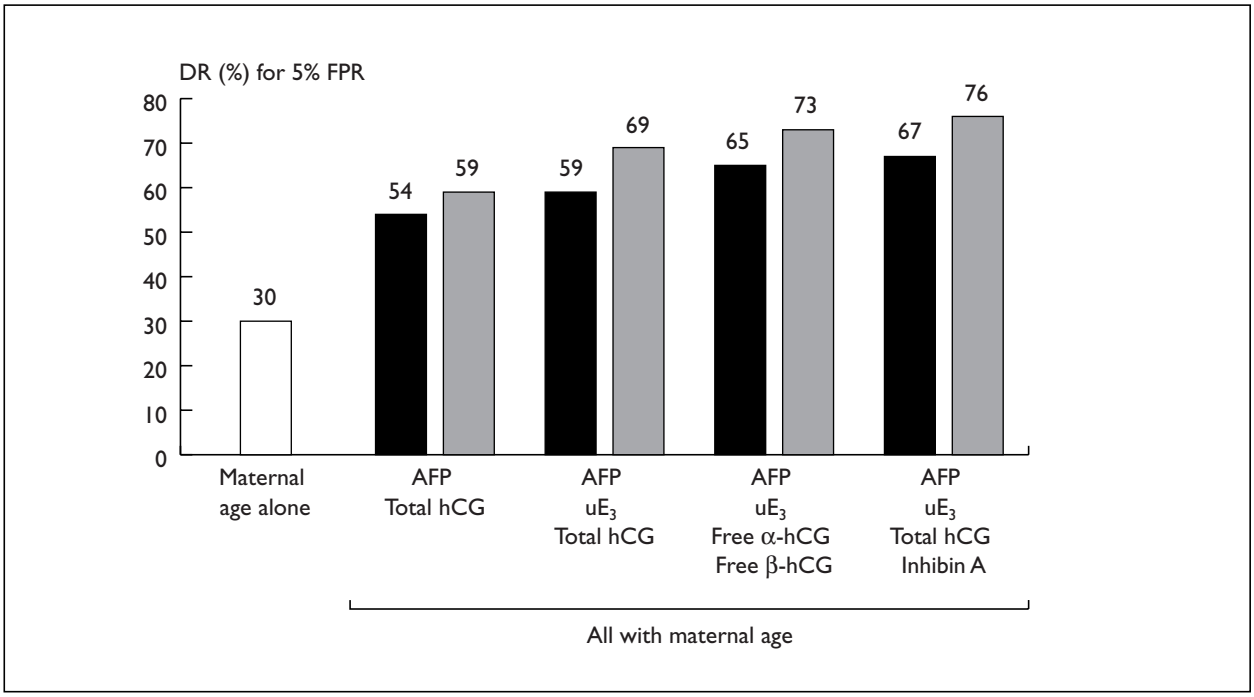

FIGURE 9 *DR for a 5% FPR according to maternal age alone and maternal age with various combinations of markers (■, gestation by dates; ■, gestation by scan)*

Factors affecting serum marker levels and screening performance

Using ultrasound scan to estimate gestational age

Ultrasound scan measurement is a useful ancillary investigation for estimating gestational age in serum screening. The standard deviation of the serum markers is smaller when a scan is used to estimate gestation than when dates are used. The effect is greatest for markers whose concentrations change most with gestational age (notably uE_3) and smallest for those that change least with gestational age (notably inhibin A) during the period of pregnancy when screening is carried out.

TABLE 5 *Estimates of FPRs according to specified DRs for various combinations of serum markers and the use of dates or scan to estimate gestational age (all results have been corrected for maternal weight)*

DR (%)	FPR (%). Maternal age with:							
	AFP and total hCG*		AFP, uE_3, hCG*		AFP, uE_3, free α-hCG, and free β-hCG		AFP, uE_3, total hCG*, and inhibin A	
	Dates	Scan	Dates	Scan	Dates	Scan	Dates	Scan
20	0.3	0.2	0.2	< 0.1	0.1	< 0.1	< 0.1	< 0.1
30	0.9	0.7	0.6	0.3	0.3	0.1	0.3	0.1
40	2.0	1.5	1.3	0.7	0.8	0.3	0.7	0.3
50	3.8	3.0	2.8	1.4	1.8	0.8	1.6	0.7
60	6.8	5.4	5.3	2.7	3.5	1.8	3.1	1.6
70	11.7	9.4	9.7	5.2	6.8	4.0	5.9	3.2
80	20.0	16.5	17.6	10.2	13.5	8.9	11.3	6.6

**Results are similar if free β-hCG is used instead of total hCG.*

Source: Screening performance estimated using statistical parameters in Wald et al, *1994;[52] 1996;[60] 1997.[61]*

TABLE 6 *Estimates of DRs according to specified DRs for various combinations of serum markers and the use of dates or scan to estimate gestational age (all results have been corrected for maternal weight)*

FPR (%)	DR (%). Maternal age with:							
	AFP and total hCG*		AFP, uE_3, hCG*		AFP, uE_3, free α-hCG, and free β-hCG		AFP, uE_3, total hCG*, and inhibin A	
	Dates	Scan	Dates	Scan	Dates	Scan	Dates	Scan
1	31	35	36	46	43	53	44	54
2	40	44	45	55	52	61	54	64
3	46	50	51	62	58	66	60	69
4	51	55	56	66	62	70	64	73
5	54	59	59	69	65	73	67	76
6	58	62	62	72	68	75	70	79
7	60	65	65	74	70	77	73	81
8	63	67	67	76	72	79	75	83
9	65	69	69	78	74	80	77	84
10	67	71	71	80	76	81	78	85

**Results are similar if free β-hCG is used instead of total hCG.*

Source: Screening performance estimated using statistical parameters in Wald et al, *1994;[52] 1996;[60] 1997.[61]*

TABLE 7 *DR and FPR at specified risk cut-off levels according to all possible one and two marker combinations of serum markers* and method of gestational age assessment (dates or scan)*

Maternal age with:	1 in 100				1 in 150				1 in 200				1 in 250				1 in 300				1 in 350			
	Dates		Scan		Dates		Scan		Dates		Scan		Dates		Scan		Dates		Scan		Dates		Scan	
	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR
AFP	18	1.0	19	1.0	23	1.8	25	1.9	29	3.1	31	3.3	34	4.4	36	4.6	37	5.7	40	6.3	41	7.1	44	8.0
uE_3	21	1.0	32	1.5	28	1.9	39	2.7	35	3.4	45	3.9	40	4.7	50	5.6	46	6.3	54	6.7	50	8.0	56	7.8
Total hCG	32	1.4	33	1.4	40	2.8	43	2.9	46	3.9	47	3.9	49	5.0	51	5.0	53	6.4	55	6.6	59	8.7	60	8.7
Free α-hCG	20	1.0	21	1.1	26	2.0	28	2.1	33	3.3	34	3.4	38	4.8	38	4.7	40	5.7	42	5.9	44	7.1	46	7.6
Free β-hCG	25	0.9	25	0.9	31	1.7	33	1.7	37	2.6	39	2.8	45	4.0	48	4.4	56	6.5	58	6.6	60	7.7	60	7.3
Inhibin A	25	1.2	25	1.2	33	2.4	33	2.4	41	4.1	41	4.1	49	6.2	49	6.2	52	7.3	52	7.3	55	8.8	55	8.8
AFP, uE_3	25	1.2	37	1.6	33	2.4	44	2.7	39	3.6	50	4.0	45	5.1	54	5.3	49	6.6	58	6.6	53	8.2	61	7.9
AFP, hCG	38	1.7	43	1.9	46	3.0	51	3.1	52	4.3	57	4.4	57	5.7	61	5.7	60	7.0	64	6.9	64	8.5	67	8.0
AFP, free α-hCG	28	1.3	30	1.4	35	2.4	37	2.5	41	3.7	43	3.7	45	5.0	47	5.0	49	6.4	51	6.5	53	7.8	54	7.8
AFP, free β-hCG	30	1.1	35	1.3	40	2.2	44	2.3	46	3.3	51	3.5	53	4.7	57	4.8	58	6.0	61	5.8	61	7.1	65	7.1
AFP, inhibin A	35	1.7	37	1.7	44	3.1	46	3.1	51	4.5	52	4.6	56	6.1	57	6.0	60	7.6	61	7.4	64	9.2	65	9.0
uE_3, hCG	39	1.5	48	1.7	46	2.6	56	2.9	52	3.9	60	3.9	56	4.9	65	5.2	59	6.1	68	6.2	62	7.3	70	7.1
uE_3, free α-hCG	35	1.4	44	1.5	43	2.4	51	2.6	48	3.4	56	3.7	53	4.9	59	4.6	56	6.0	62	5.7	59	7.0	65	7.0
uE_3, free β-hCG	36	1.1	46	1.3	44	2.1	53	2.2	51	3.2	57	3.1	54	4.2	61	4.2	59	5.6	64	4.9	61	6.6	66	5.9
uE_3, inhibin A	39	1.6	46	1.7	47	2.8	54	2.9	54	4.3	59	4.0	58	5.4	64	5.4	61	6.6	66	6.3	65	8.2	68	7.2
hCG, inhibin A	41	1.7	43	1.8	49	3.0	51	2.9	55	4.2	56	4.1	59	5.5	61	5.4	63	6.7	64	6.6	66	8.0	67	7.8
Free α-hCG, hCG	35	1.5	36	1.5	42	2.6	44	2.7	48	3.9	49	3.9	51	5.0	53	5.0	55	6.3	57	6.4	58	7.5	59	7.6
Free α-hCG, free β-hCG	32	1.0	32	1.1	40	2.0	41	2.0	47	3.1	47	3.0	51	4.1	52	4.2	57	5.6	57	5.4	59	6.6	60	6.6
Free α-hCG, inhibin A	33	1.5	34	1.5	42	2.8	42	2.8	47	4.0	48	4.0	52	5.5	53	5.5	56	6.9	57	6.8	59	8.1	60	8.1
Free β-hCG, inhibin A	37	1.3	39	1.3	46	2.4	47	2.3	53	3.6	54	3.6	57	4.7	59	4.8	61	5.9	62	5.8	65	7.2	65	7.0

* *Excluding combinations that include both hCG and free β-hCG.*

Marker levels are adjusted for maternal weight.

The OAPR can be obtained by applying the likelihood ratio (LR) (LR = DR/FPR) to the birth prevalence of Down's syndrome (1.3 per 1000 expressed as an odds is 1:768); OAPR = 1:768/LR.

TABLE 8 *DR and FPR at specified risk cut-off levels according to all possible three marker combinations of serum markers* and method of gestational age assessment (dates or scan)*

Maternal age with:	1 in 100				1 in 150				1 in 200				1 in 250				1 in 300				1 in 350			
	Dates		Scan		Dates		Scan		Dates		Scan		Dates		Scan		Dates		Scan		Dates		Scan	
	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR
AFP, uE_3, hCG	43	1.7	54	1.8	50	2.8	61	2.9	55	3.9	66	3.9	60	5.1	69	4.9	63	6.3	72	5.9	66	7.5	74	6.8
AFP, uE_3, free α-hCG	38	1.4	48	1.6	46	2.5	55	2.6	51	3.6	59	3.5	55	4.8	63	4.6	59	6.0	66	5.5	62	7.1	68	6.6
AFP, uE_3, free β-hCG	38	1.3	50	1.4	47	2.3	57	2.2	53	3.4	62	3.1	58	4.5	65	4.1	61	5.6	68	4.9	64	6.7	71	5.8
AFP, uE_3, inhibin A	43	1.7	52	1.8	51	3.0	59	2.8	57	4.2	64	3.9	61	5.4	67	4.9	65	6.7	70	5.9	68	7.8	73	6.9
AFP, hCG, free α-hCG	41	1.6	45	1.8	48	2.8	52	2.9	54	4.0	57	4.1	58	5.3	61	5.3	61	6.5	65	6.5	65	7.8	68	7.7
AFP, hCG, inhibin A	48	1.9	53	1.9	56	3.1	60	3.1	61	4.3	65	4.2	66	5.5	69	5.3	69	6.7	72	6.3	71	7.7	74	7.3
AFP, free α-hCG, inhibin A	42	1.7	43	1.7	50	2.9	51	2.9	55	4.1	57	4.2	60	5.4	61	5.3	63	6.7	64	6.6	66	7.9	67	7.9
AFP, free β-hCG, inhibin A	43	1.5	48	1.5	52	2.6	56	2.6	59	3.7	61	3.6	63	4.8	66	4.7	67	6.0	69	5.6	70	7.0	72	6.6
AFP, free α-hCG, free β-hCG	38	1.2	41	1.3	46	2.2	49	2.3	53	3.3	55	3.3	57	4.3	60	4.5	61	5.5	64	5.6	65	6.6	67	6.5
uE_3, hCG, free α-hCG	43	1.5	51	1.6	50	2.5	58	2.6	55	3.5	62	3.6	59	4.6	66	4.6	61	5.6	69	5.6	64	6.7	71	6.5
uE_3, hCG, inhibin A	48	1.7	56	1.7	55	2.8	63	2.8	59	3.8	67	3.7	64	5.0	70	4.7	66	5.9	73	5.6	69	7.0	75	6.4
uE_3, free α-hCG, inhibin A	46	1.6	53	1.6	53	2.7	59	2.6	58	3.7	64	3.5	62	4.8	67	4.5	65	5.8	70	5.4	68	6.8	72	6.3
uE_3, free β-hCG, inhibin A	46	1.4	53	1.4	53	2.4	59	2.3	58	3.4	64	3.2	62	4.5	67	4.0	65	5.5	70	4.9	68	6.5	72	5.7
uE_3, free α-hCG, Free β-hCG	43	1.2	52	1.3	50	2.1	58	2.1	56	3.1	62	2.9	59	4.0	65	3.8	63	5.1	67	4.6	65	6.1	70	5.5
Inhibin A, hCG, free α-hCG	43	1.7	44	1.7	50	2.8	51	2.8	55	4.0	57	4.0	60	5.2	61	5.2	63	6.4	64	6.4	66	7.6	67	7.6
Free α-hCG, free β-hCG, inhibin A	41	1.3	42	1.3	49	2.3	50	2.3	55	3.4	56	3.4	59	4.5	60	4.5	63	5.6	64	5.6	66	6.6	66	6.6

* *Excluding combinations that include both hCG and free β-hCG.*

Marker levels are adjusted for maternal weight.

The OAPR can be obtained by applying the LR (LR = DR/FPR) to the birth prevalence of Down's syndrome (1.3 per 1000 expressed as an odds is 1:768); OAPR = 1:768/LR.

TABLE 9 *DR and FPR at specified risk cut-off levels according to all possible four marker combinations of serum markers* and method of gestational age assessment (dates or scan)*

Maternal age with:	1 in 100				1 in 150				1 in 200				1 in 250				1 in 300				1 in 350			
	Dates		Scan		Dates		Scan		Dates		Scan		Dates		Scan		Dates		Scan		Dates		Scan	
	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR	DR	FPR
AFP, uE_3, hCG, inhibin A	52	1.8	62	1.7	59	2.8	68	2.7	64	3.9	72	3.5	67	5.0	75	4.4	70	6.0	77	5.2	73	7.0	79	5.9
AFP, uE_3, free α-hCG, inhibin A	50	1.6	57	1.6	57	2.6	63	2.5	61	3.7	67	3.4	65	4.7	70	4.2	68	5.7	73	5.1	70	6.7	75	5.9
AFP, uE_3, free β-hCG, inhibin A	49	1.5	58	1.4	57	2.5	64	2.3	62	3.5	69	3.1	66	4.6	72	3.9	69	5.6	74	4.7	71	6.5	76	5.5
AFP, uE_3, hCG, free α-hCG	47	1.6	56	1.6	54	2.6	62	2.6	58	3.6	67	3.5	62	4.7	70	4.4	65	5.7	72	5.3	68	6.8	75	6.2
AFP, uE_3, free α-hCG, free β-hCG	46	1.3	56	1.3	53	2.2	62	2.1	59	3.2	66	2.9	63	4.1	69	3.7	66	5.1	71	4.5	68	6.1	73	5.2
AFP, inhibin A, hCG, free α-hCG	49	1.8	53	1.8	57	2.9	60	2.9	62	4.1	65	4.0	66	5.2	69	5.1	69	6.3	72	6.1	72	7.3	74	7.1
AFP, free α-hCG, free β-hCG, inhibin A	47	1.4	51	1.5	55	2.4	58	2.4	61	3.4	63	3.4	65	4.4	67	4.4	68	5.5	70	5.3	71	6.4	73	6.2
uE_3, inhibin A, hCG, free α-hCG	50	1.6	58	1.6	57	2.6	64	2.5	61	3.6	68	3.4	65	4.6	71	4.3	68	5.5	73	5.2	70	6.5	75	6.0
uE_3, free α-hCG, free β-hCG, inhibin A	50	1.4	57	1.4	57	2.3	63	2.2	61	3.2	66	3.0	65	4.1	69	3.8	68	5.0	72	4.5	70	6.0	74	5.3

* *Excluding combinations that include both hCG and free β-hCG.*

Marker levels are adjusted for maternal weight.

The OAPR can be obtained by applying the LR (LR = DR/FPR) to the birth prevalence of Down's syndrome (1.3 per 1000 expressed as an odds is 1:768); OAPR = 1:768/LR.

TABLE 10 *Effect on the standard deviation of serum marker levels of using scan compared with dates to estimate gestational age*

Serum marker	Standard deviation			
	Down's syndrome pregnancies		Unaffected pregnancies	
	Dates	Scan	Dates	Scan
AFP	0.1965	0.1821	0.1936	0.1789
uE_3	0.1462	0.1210	0.1374	0.1102
Total hCG	0.2606	0.2520	0.2336	0.2239
Free α-hCG	0.1731	0.1666	0.1473	0.1396
Free β-hCG	0.2999	0.2963	0.2424	0.2379
Inhibin A	0.1986	0.1986	0.2154	0.2154

Marker levels are adjusted for maternal weight.

Source: Wald et al, *1994;*[52] *1996;*[60] *1997.*[61]

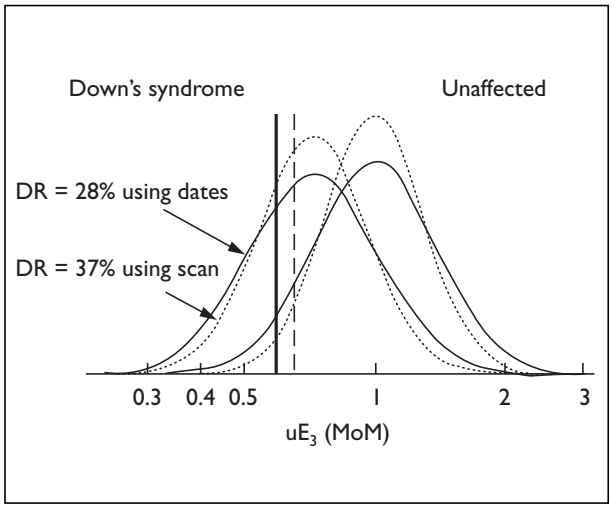

FIGURE 10 *Distribution of uE_3 (MoM) in affected and unaffected pregnancies using dates (——) or ultrasound scan (·····) to estimate gestational age. The DRs for a 5% FPR are indicated. The vertical lines represent a 5% FPR using dates (——) or scan (– – –)*

Table 10 shows estimates of the standard deviations of the serum markers using dates or scan to estimate gestational age. *Figure 10* shows the distribution of uE_3 in Down's syndrome and unaffected pregnancies using dates and scan to estimate gestational age, illustrating how the extent of overlap between the two distributions is less if scan is used, and hence the improved screening performance. *Figure 9* shows the effects on screening of various combinations of serum markers.

Maternal weight

Serum AFP, uE_3, and hCG concentrations change with maternal weight. A summary of the literature *(Table 11)* shows that, on average, for a 20 kg increase in weight serum AFP decreases by about 17%, uE_3 decreases by about 7%, and hCG decreases by about 16%. Methods to adjust for maternal weight have been described by Watt *et al*[92] and Neveux *et al*,[93] both of whom obtained similar results. *Table 12* shows the effect on screening performance if the MoM values for each marker are adjusted for maternal weight. Although the improvement is small, the adjustment is simple to perform and therefore worthwhile.

Insulin-dependent diabetes

Some serum marker levels are, on average, lower in women with insulin-dependent diabetes. *Table 13* shows the published literature on studies according to whether the serum markers were corrected for maternal weight or not. The table shows the median value for each marker and the weighted average for all studies. After weight correction, AFP is about 10% lower in these women, uE_3 is 7% lower, free α-hCG is 11% lower, and inhibin A is 9% lower. The differences are statistically significant and can therefore usefully be taken into account in screening. There were no significant differences for total hCG and free β-hCG. Adjustment for diabetes is carried out by dividing the observed MoM for a woman with diabetes by the corresponding median MoM in diabetic women without Down's syndrome pregnancies *(Table 13)*. The risk based on such an adjusted MoM value is termed a 'pseudo risk', because it is not the true risk, the calculation of which would require data on the distributions of the markers among insulin-dependent diabetic women with Down's syndrome pregnancies. This adjustment allows women to be classified as screen positive or screen negative in a way that will keep the false-positive rate in diabetic women the same as in non-diabetic women. Because the risk estimate is not a true risk estimate, it is not reported; the women are simply classified as either screen positive or negative.

Twin pregnancies

Serum marker levels in women with twin pregnancies might be expected to be about twice those in singleton pregnancies. This is, in fact, observed, although some markers have levels a little greater (for example, AFP) and some a little less (for example, uE_3), as shown in *Table 14*. As with diabetic women, a 'pseudo risk' is calculated by dividing the observed MoM value in a twin pregnancy by the median MoM value in twin pregnancies without Down's syndrome. This is not a true risk but, again, it will have the effect of keeping the false-positive rate in twin pregnancies the same as that in

TABLE 11 *Median serum marker levels according to maternal weight*

Study	Maternal weight (kg)	Number of women	Median MoM for		
			AFP	uE_3	hCG
Wald *et al,* 1981[86]	< 45	17	1.33	–	–
	45–54	182	1.20	–	–
	55–64	430	1.08	–	–
	65–74	192	0.95	–	–
	75–84	60	0.84	–	–
	≥ 85	21	0.79	–	–
% decrease for a 20 kg increase in weight		**902**	**20%**		
Haddow *et al,* 1981[87]	< 50 (approx)	129	1.20	–	–
	50–64	925	1.10	–	–
	65–77	431	1.00	–	–
	> 77	159	0.80	–	–
% decrease for a 20 kg increase in weight		**1644**	**16%**		
Macri *et al,* 1986[88]	< 50 (approx)	–	1.23	–	–
	50–64	–	1.08	–	–
	65–77	–	0.96	–	–
	> 77	–	0.83	–	–
% decrease for a 20 kg increase in weight		**5740**	**16%**		
Reynolds *et al,* 1991[89]	≤ 50	113 (10)*	1.37	0.98	1.36
	51–60	520 (60)	1.30	0.95	1.04
	61–70	431 (74)	1.08	0.95	1.06
	71–80	194 (25)	0.94	0.89	1.00
	81–90	84 (18)	0.85	0.93	0.95
	> 90	66 (10)	0.83	0.94	0.75
% decrease for a 20 kg increase in weight		**1408 (197)**	**22%**	**2%**	**13%**
Wald *et al,* 1992[90]	< 50	172	1.09	1.09	1.13
	50–54	215	1.12	1.04	1.16
	55–59	366	1.04	0.99	1.11
	60–64	346	0.99	0.99	1.04
	65–69	290	0.93	0.95	0.92
	70–74	165	0.84	0.99	0.91
	75–79	129	0.83	0.89	1.00
	≥ 80	206	0.80	0.87	0.82
% decrease for a 20 kg increase in weight		**1889**	**16%**	**9%**	**15%**
Bartels *et al,* 1993[91]	< 45	20	1.21	1.19	1.32
	45–54	527	1.19	1.04	1.13
	55–64	1987	1.06	0.98	1.06
	65–74	1608	0.96	0.94	0.96
	75–84	645	0.90	0.94	0.87
	85–94	220	0.80	0.91	0.85
	≥ 95	142	0.71	0.86	0.69
% decrease for a 20 kg increase in weight		**5149**	**18%**	**6%**	**17%**
All studies†	–	–	**17%**	**7%**	**16%**
% decrease for a 20 kg increase in weight		**16,732**			

* *The numbers in brackets refer to the number of women used for the analysis of uE_3.*

† *Obtained by first performing a regression of the median MoM (in logarithms) with the midpoint of each maternal weight group and weighted by the number of women in each weight group. The regression slopes are then pooled across studies, weighted by the total number of women in the study.*

TABLE 12 *Effect on screening performance of correcting serum marker levels for maternal weight*

Maternal age with:	Gestational age estimated by			
	Dates		Scan	
	Weight correction		Weight correction	
	No	Yes	No	Yes
AFP, hCG				
DR (%) for 5% FPR	54	54	58	59
FPR (%) for 60% DR	6.9	6.8	5.7	5.4
AFP, uE_3, hCG				
DR (%) for 5% FPR	59	59	67	69
FPR (%) for 60% DR	5.4	5.3	3.0	2.7
AFP, uE_3, free α-hCG, free β-hCG				
DR (%) for 5% FPR	65	65	72	73
FPR (%) for 70% DR	7.0	6.8	4.4	4.0
AFP, uE_3, hCG, inhibin A				
DR (%) for 5% FPR	67	67	75	76
FPR (%) for 70% DR	6.0	5.9	3.5	3.2

Source: Screening performance estimated using statistical parameters in Wald et al, *1994,[52] 1996,[60] 1997,[61] and the method described in Watt* et al, *1996.[92]*

singleton pregnancies. A woman may be classified as screen positive or screen negative using the same cut-off level used for singleton pregnancies but without reporting a risk estimate.

Screening in twin pregnancies poses a problem because of the presence of two foetuses and the possibility that only one may be affected. There is a reasonable reluctance to act on a positive screening result and perform an amniocentesis on a twin pregnancy, or consider a termination of an unaffected co-twin in the event of a positive diagnosis. The discovery of a twin pregnancy may therefore be seen as an indication to avoid screening or further diagnostic tests.

Ethnic origin

Table 15 shows published estimates of the ratio of the median marker MoM values in black (Afro-Caribbean) women compared with white women in studies according to whether serum marker levels were corrected for maternal weight. AFP, total hCG, and inhibin A show the greatest effect; the other markers were similar in both black and white women. *Table 16* shows, in a similar way, the median marker levels in South Asian women. In calculating risk for a black or South Asian woman, the observed MoMs can be adjusted by the method of Watt and colleagues.[92] The effect on screening performance is small – the detection rate increases by about 0.5% for a false-positive rate of 5%. The adjustment is worthwhile because it does not require resources and because of its established value in screening for open NTDs using serum AFP,[125] where the false-positive rate in black women is about 2.5 times that in white women for a fixed AFP cut-off level. In South Asian women, the false-positive rate is about two-thirds of that in white women. Adjusting for ethnic origin in Down's syndrome screening will tend to equalise the false-positive rate among women of different ethnic groups.

Smoking

Serum marker levels tend to be different in women who smoke compared with women who do not. *Table 17* shows the effect of smoking status on the median MoM for AFP, uE_3, total hCG, and free β-hCG. Only one study corrected for maternal weight. The greatest difference is for total hCG. Smokers have, on average, levels about 18% lower. The effect on the other markers is small. On average, AFP is about 3% higher, uE_3 is 4% lower, total hCG is 18% lower, and free β-hCG is 6% lower for smokers than for non-smokers. Adjusting serum marker levels for smoking status has a very small effect on screening performance; at a given false-positive rate the detection rate increases by less than 1%. Adjustment for smoking status in a manner similar to adjusting for twins or diabetes assumes that the birth prevalence of Down's syndrome is similar in smoking and non-smoking women. Cuckle and colleagues[136] showed that the birth prevalence is about 16% lower in smoking women. This might be due to a higher miscarriage rate in smoking women. At present, because of the small effect on screening performance and the uncertainty over the difference in birth prevalence in smokers, there is insufficient evidence to warrant adjustment for smoking status.

Number of previous pregnancies

Table 18 shows the effect of the number of previous pregnancies on serum marker levels. All studies are corrected for maternal weight. Total hCG is negatively associated with parity, decreasing by about 3% for each previous birth. The reason for this decline is not known. The effect on screening performance of adjusting hCG levels is negligible. At a false-positive rate of 5%, the detection rate would increase by only 0.1%. It is, therefore, not worthwhile adjusting MoM values for the number of previous pregnancies.

TABLE 13 *Median serum marker levels in pregnant women with and without insulin-dependent diabetes mellitus*

Serum marker and study	Number of women		Median MoM in diabetic women	
	Non-diabetic	Diabetic	Not weight corrected	Weight corrected
AFP				
Reece *et al,* 1987[94]	–	161*	0.91	1.04
Greene *et al,* 1988[95]	–	164	0.81	0.81
Canick *et al,* 1990[96]	4711	24	–	0.97
Sunderji *et al,* 1992[97]	–	132	–	0.84
Henriques *et al,* 1993[98]	–	151	0.78	–
Palomaki *et al,* 1994[99]	20,269	52	–	0.74
Selby *et al,* 1995[100]	66	37	–	0.88
Crossley *et al,* 1996[101]	15,365	234	0.89	0.98
Wald *et al,* 1992;[102] 1996[103]	252	126	0.77	0.82
Weighted geometric mean†	**40,663**	**1081**	**0.84‡**	**0.90‡**
uE_3				
Canick *et al,* 1990[96]	4711	24	–	0.87‡
Palomaki *et al,* 1994[99]	20,269	52	–	0.94
Wald *et al,* 1992;[102] 1996[103]	252	126	0.92	0.94
Weighted geometric mean†	**25,232**	**202**	**0.92‡**	**0.93‡**
Total hCG				
Canick *et al,* 1990[96]	4711	24	–	0.87
Palomaki *et al,* 1994[99]	20,269	52	–	0.96
Selby *et al,* 1995[100]	66	37	–	1.17
Crossley *et al,* 1996[101]	15,365	234	0.91	0.92
Wald *et al,* 1992;[102] 1996[103]	252	126	0.95	1.00
Weighted geometric mean†	**40,663**	**473**	**0.92**	**0.96**
Free α-hCG				
Wald *et al,* 1994;[104] 1996[103]	251	126	0.86‡	0.89‡
Free β-hCG				
Wald *et al,* 1994;[104] 1996[103]	251	126	0.96	1.01
Inhibin A				
Wald *et al,* 1996[103]	250	126	0.88‡	0.91‡

** 129 had values which were weight corrected.*
† The pooled median MoM was calculated by weighting the log median MoM for each study by the corresponding number of diabetic women.
‡ Indicates that the difference between non-diabetic and diabetic pregnancies was statistically significant.

Recurrence risk of a Down's syndrome pregnancy

The risk of having a second affected pregnancy will be greater than if there is no previous history. The increase in risk is additive to the age-specific risk and will depend on whether the foetus in the first affected pregnancy had a non-inherited or inherited case of Down's syndrome. If it was non-inherited, the recurrence risk at term is the age-specific risk plus 0.34%, estimated from a review of three studies on the recurrence risk of non-inherited Down's syndrome.[142] For example, the recurrence risk in a woman aged 35 years is 1 in 174 (based on her occurrence age-specific risk of 1 in 425 plus 0.34%, that is 1 in $1/((1/425) + 0.34\%)$. If the previous affected pregnancy was an inherited case, the increase in risk would be greater, by about 10%, and genetic counselling would be required.

Routine repeat testing

Serum marker levels fluctuate over time in a given pregnancy and therefore contribute to the overall variance. The effect on screening performance of routinely taking a second blood sample about one week after the first has been investigated for AFP, uE_3, total hCG, free α-hCG, and free β-hCG.[143,144] When the quadruple test (AFP, uE_3, free α- and β-hCG) is used, the detection rate for a 5% false-positive rate is 69% if all women have repeat tests compared with 65% if none have repeat tests. The increase is less if any type of selective retesting policy is used, while maintaining a constant false-positive rate. Any repeat testing would incur the extra costs of recalling women, the extra assays, and the extra counselling after both test results. There is increased anxiety while waiting for a second test result. In general, women should not be offered repeat tests but, if one is performed, it is important that the second test is interpreted in the light of the previous one to avoid the inaccurate risk estimation that can arise on account of regression to the mean.

Women who were screen positive in a previous pregnancy

Two studies[145,146] have shown that a woman is more likely to be screen positive if she was screen positive during a previous pregnancy, and the extent of this association will depend on her age.[145,146] A woman aged 20 years will be three to five times more likely to be screen positive in a later pregnancy if she was screen positive in a previous pregnancy, whereas a woman aged 40 years will be 1.4 times more likely to be screen positive.[145,146] However, until more data are available on affected pregnancies, it is not possible to adjust risks to allow for the result of a previous pregnancy.

Assisted reproduction (ovulation induction and *in vitro* fertilisation)

There is a suggestion that serum marker levels are affected by assisted reproduction using either ovulation induction or *in vitro* fertilisation.[147–149] In women who had ovulation induction, hCG levels were, on average, 9% higher and uE_3 levels were 8% lower.[147] There was no difference for AFP. In women who had *in vitro* fertilisation a weighted geometric mean of marker levels in three studies[147–149] showed that there was no significant effect on AFP levels (median MoM 0.96) or hCG levels (median MoM 0.99), though there was a suggestion that uE_3 levels were low (median MoM 0.92) in one study.[147] Future research will, no doubt, clarify the position. At present, the effect on marker levels is insufficient to warrant adjustment in risk estimation.

TABLE 14 *Median serum marker levels in unaffected singleton and twin pregnancies*

Serum marker and study	Number of women		Median MoM
	Singleton	Twin	Twin
AFP			
Knight *et al,* 1981[105]	–	37	2.10
Ghosh *et al,* 1982[106]	–	219	2.50
Librach *et al,* 1984[107]	–	47	≈2.00
Walker & Patel, 1986[108]	–	166	2.20
Alpert *et al,* 1990[109]	320	51	1.58
Canick *et al,* 1990[96]	2700	35	2.32
Johnson *et al,* 1990[110]	13,304	138	≈2.50
Dar *et al,* 1991[111]	247	126	2.13
Nieb *et al,* 1991[112]	–	43	2.23
Wald *et al,* 1991[113]	600	200	2.13
Spencer *et al,* 1994[114]	6661	420	2.28
Neveux *et al,* 1996[115]	34,740	410	2.16
Weighted geometric mean†	**58,572**	**1892**	**2.23***
uE_3			
Alpert *et al,* 1990[109]	320	51	1.44
Canick *et al,* 1990[96]	2700	35	1.67
Nieb *et al,* 1991[112]	–	43	1.28
Wald *et al,* 1991[113]	600	200	1.67
Neveux *et al,* 1996[115]	34,740	410	1.72
Weighted geometric mean†	**38,360**	**739**	**1.65***
Total hCG			
Alpert *et al,* 1990[109]	320	51	1.80
Canick *et al,* 1990[96]	2700	35	1.93
Bogart *et al,* 1991[116]	3140	33	1.48
Dar *et al,* 1991[111]	247	247	2.00
Nebiolo *et al,* 1991[117]	983	192	1.96
Nieb *et al,* 1991[112]	–	43	2.43
Wald *et al,* 1991[113]	600	200	1.84
Neveux *et al,* 1996[115]	34,740	410	2.17
Weighted geometric mean†	**42,730**	**1211**	**2.01***
Free α-hCG			
Wald & Densem, 1994[118]	600	199	1.66*
Free β-hCG			
Spencer *et al,* 1994[114]	6661	420	2.17
Wald & Densem, 1994[119]	600	199	1.90
Weighted geometric mean†	**7261**	**619**	**2.08***
Inhibin A			
Watt & Wald, 1996[120]	600	199	1.99*

* *Indicates the difference between singleton and twin pregnancies was statistically significant.*

† *The pooled median MoM was calculated by weighting the log median MoM for each study by the corresponding number of singleton or twin pregnancies.*

TABLE 15 *Second trimester Down's syndrome serum markers in black women compared with white women*

Serum marker and study	Number of white women		Number of black women		Ratio of median MoM in black women to median MoM in white women	
	Weight corrected	Not weight corrected	Weight corrected	Not weight corrected	Weight corrected	Not weight corrected
AFP						
Macri *et al,* 1976[121]	–	59	–	46	–	0.95
Crandall *et al,* 1983[122]	–	6544	–	439	–	1.10
Johnson, 1985[123]	939	–	431	–	1.18	–
Baumgarten, 1986[124]	–	39,919	–	2788	–	1.13
Wald & Cuckle, 1987[125]	4525	–	36	–	1.10	–
Canick *et al,* 1990[96]	4500	–	235	–	1.12	–
Watt *et al,* 1996[92]	9462	9462	4215	4215	1.22	1.20
O'Brien *et al,* 1997[126]	140,532	–	40,240	–	1.14	–
Weighted geometric mean†	**159,958**	**55,984**	**45,157**	**7488**	**1.15***	**1.17***
uE$_3$						
Simpson *et al,* 1990[127]	–	565	–	599	–	0.95
Canick *et al,* 1990[96]	4500	–	235	–	0.95	–
Burton & Nieb, 1991[128]	–	1311	–	1365	–	1.04
Kulch *et al,* 1993[129]	268	268	134	134	1.05	0.97
Watt *et al,* 1996[92]	9462	9462	4215	4215	1.00	1.00
O'Brien *et al,* 1997[126]	–	40,197	–	6765	–	0.97
Weighted geometric mean†	**14,230**	**51,803**	**4584**	**13,078**	**1.00**	**0.99**
Total hCG						
Simpson *et al,* 1990[127]	–	578	–	603	–	1.21
Canick *et al,* 1990[96]	4500	–	235	–	1.03	–
Muller & Boué, 1990[130]	–	1894	–	214	–	1.27
Bogart *et al,* 1991[116]	2505	–	310	–	1.10	–
Burton & Nieb, 1991[128]	–	1311	–	1365	–	1.06
Kulch *et al,* 1993[129]	268	268	134	134	1.19	1.16
Watt *et al,* 1996[92]	9462	9462	4215	4215	1.19	1.15
O'Brien *et al,* 1997[126]	–	85,937	–	21,491	–	1.11
Weighted geometric mean†	**16,735**	**99,450**	**4894**	**28,022**	**1.18***	**1.12***
Free α-hCG						
Watt *et al,* 1996[92]	922	922	449	449	0.92	0.92
Free β-hCG						
Watt *et al,* 1996[92]	922	922	449	449	1.12*	1.09*
Inhibin A						
Watt *et al,* 1996[92]	922	922	449	449	0.92*	0.89*

* *Indicates that the ratio was statistically significant.*

† *Weighted by numbers of black women.*

TABLE 16 *Second trimester AFP levels in South Asian women compared with white women*

Serum marker and study	Number of women				Ratio of median MoM in South Asian women to median MoM in white women	
	White		South Asian			
	Weight corrected	Not weight corrected	Weight corrected	Not weight corrected	Weight corrected	Not weight corrected
AFP						
Shapiro *et al,* 1975[131]	–	24	–	24	–	0.57
Cuckle *et al,* 1987[132]	4231	26,818	80	531	0.93	0.94
Watt *et al,* 1996[92]	9462	9462	4392	4392	0.94	1.03
Weighted geometric mean[†]	**13,693**	**36,304**	**4472**	**4947**	**0.94**	**1.02**
uE$_3$						
Watt *et al,* 1996[92]	9459	9459	4391	4391	1.07[*]	1.11
Total hCG						
Watt *et al,* 1996[92]	9459	9459	4391	4391	1.06[*]	1.12
Free α-hCG						
Watt *et al,* 1996[92]	922	922	135	135	1.03	1.11
Free β-hCG						
Watt *et al,* 1996[92]	922	922	135	135	0.91	0.99
Inhibin A						
Watt *et al,* 1996[92]	922	922	135	135	1.01	1.09

[*] *Indicates that the ratio was statistically significant.*
[†] *Weighted by the number of South Asian women.*
The MoM values in all studies were not adjusted for maternal weight.

TABLE 17 *Median serum marker levels in smoking and non-smoking pregnant women*

Serum marker and study	Number of women		Median MoM	
	Non-smokers	Smokers	Non-smokers	Smokers
AFP				
Cuckle *et al,* 1990[133]	319	66	0.99	1.06
Palomaki *et al,* 1993[134]	18,339	5294	1.00	1.03
Bartels *et al,* 1993[91]	4131	1018	0.99	1.04
Spencer, 1993[135†]	–	–	0.99	1.03
Weighted geometric mean[*]	**> 22,789**	**> 6378**	**1.00**	**1.03**
uE$_3$				
Cuckle *et al,* 1990[133]	319	66	1.00	0.95
Bartels *et al,* 1993[91]	4131	1018	0.98	0.95
Palomaki *et al,* 1993[134]	18,339	5294	0.99	0.96
Weighted geometric mean[*]	**22,789**	**6378**	**0.99**	**0.96**
Total hCG				
Cuckle *et al,* 1990[133]	319	66	1.02	0.91
Bartels *et al,* 1993[91]	4131	1018	1.04	0.83
Palomaki *et al,* 1993[134]	18,339	5294	1.07	0.82
Weighted geometric mean[*]	**22,789**	**6378**	**1.06**	**0.82**
Free β-hCG				
Spencer, 1993[135†]	–	–	1.01	0.94

[*] *The pooled median MoM was calculated by weighting the log median MoM for each study by the corresponding number of women who smoked.*
[†] *The number of non-smokers and smokers was unspecified – there were 1000 in total.*
The MoM values in all studies, except for AFP in Palomaki et al, *1993,*[134] *were not adjusted for maternal weight.*

TABLE 18 *Effect of the number of previous pregnancies on serum marker levels in the second trimester of pregnancy*

Serum marker and study	No. of women	Median MoM according to number of previous pregnancies*					Statistical significance
		0	1	2	3	≥ 4	
AFP							
Haddow *et al,* 1995[137]	16,675	1.01	1.00	1.00	0.97	1.00	NS
Zimmermann *et al,* 1995[138]	1114	1.09	←	1.04	→	→	$p < 0.05$
Wald & Watt, 1996[139]	16,666	1.01	0.99	1.02	0.98	1.04	NS
Spencer, 1995[140]	4058	1.01	1.01	0.98	1.02	1.00	NS
uE_3							
Haddow *et al,* 1995[137]	16,675	1.01	0.99	1.00	0.99	0.99	NS
Zimmermann *et al,* 1995[138]	1114	1.07	←	1.02	→	→	Yes
Wald & Watt, 1996[139]	16,666	1.01	0.98	0.99	1.00	1.04	NS
Barkai *et al,* 1996[141]	16,218	1.02	1.00	0.97	0.98	0.97	$p < 0.005$
Total hCG							
Haddow *et al,* 1995[137]	16,675	1.04	0.98	0.95	0.93	0.86	$p < 0.001$
Zimmermann *et al,* 1995[138]	1114	1.17	←	1.14	→	→	NS
Wald & Watt, 1996[139]	16,666	1.04	1.01	0.96	0.94	0.92	$p = 0.006$
Barkai *et al,* 1996[141]	22,335	1.04	0.98	0.97	0.99	0.98	NS
Free α-hCG							
Wald & Watt, 1996[139]	693	1.00	0.97	1.07	0.99	0.99	NS
Free β-hCG							
Spencer, 1995[140]	4058	–	1.05	1.01	1.00	0.99	$p < 0.05$
Wald & Watt, 1996[139]	693	1.00	0.97	1.03	1.00	1.04	NS
Inhibin A							
Wald & Watt, 1996[139]	693	1.02	0.97	1.04	1.04	1.09	NS

NS = not statistically significant (p > 0.05).

* *Most studies looked at parity (the number of previous pregnancies beyond 28 weeks' gestation). One study (Barkai[141]) looked at gravidity (the total number of previous pregnancies, including miscarriages).*

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Chapter 4

Urinary markers and foetal cells in maternal blood

Urinary markers

Levels of hCG and its subunits are raised in maternal serum in Down's syndrome pregnancies, and it has been shown that hCG and free β-hCG levels are also raised in maternal urine.[1,2] *Figure 11* shows the median MoM for urinary β-core hCG (or urine β-core fragment), a breakdown product of hCG, in affected pregnancies. The pooled median is 3.67 MoM, suggesting that β-core hCG is a potentially effective marker. When β-core hCG is combined with maternal age, estimates of the predicted detection rate for a 5% false-positive rate range from 41% to 80%.[3,4,7] Larger studies, based on samples collected from women receiving routine antenatal care, are required to assess the performance of this marker on its own and possibly with other urinary and serum markers. *Table 19* shows other urinary markers of interest during the first and second trimesters. Free β-hCG and total oestriol seem to be potentially useful markers.

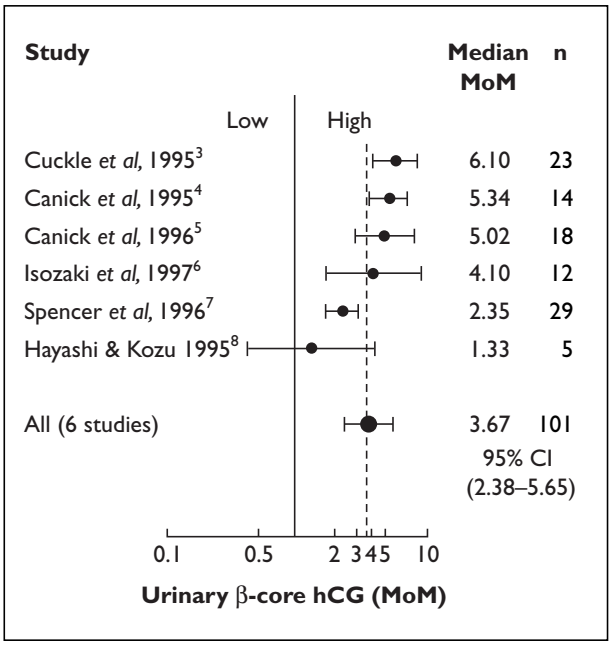

FIGURE 11 *Median (or mean) urinary β-core hCG (adjusted for creatinine concentration) in Down's syndrome pregnancies at 15–22 weeks of pregnancy with 95% CI and the number of affected pregnancies in each study. The pooled median is indicated by the vertical broken line*

TABLE 19 *Urinary markers in Down's syndrome pregnancies (excluding β-core hCG at 15–22 weeks)*

Urinary marker/ study	No. of Down's syndrome pregnancies	Median MoM	95% CI
10–14 weeks:			
Urinary β-core hCG			
Kornman *et al*, 1997[9]	5	1.30	0.27–6.25
Macintosh *et al*, 1997[10]	9	1.16	0.47–2.88
Spencer *et al*, 1997[1]	22	2.91	0.85–9.94
All (3 studies)	**36**	**1.51**	**0.90–2.54**
Free β-hCG			
Spencer *et al*, 1997[1]	22	1.81	0.87–3.75
Total oestriol			
Spencer *et al*, 1997[1]	22	0.83	0.67–1.02
15–22 weeks:			
Total oestrogen			
Cuckle *et al*, 1995[3]	23	0.74	0.54–1.02
Total oestriol			
Kellner *et al*, 1996[11]	32	0.64	0.51–0.80
Free α-hCG			
Cuckle *et al*, 1995[3]	23	1.07	0.66–1.74
Free β-hCG			
Spencer *et al*, 1996[7]	29	2.47	1.66–3.68
Kellner *et al*, 1996[2]	14	2.61	1.45–4.72
All (2 studies)	**43**	**2.51**	**1.98–3.21**
Total hCG			
Kellner *et al*, 1996[2]	14	2.14	1.11–4.11

Foetal cells in maternal blood

The isolation of foetal cells in maternal blood has been proposed as a possible screening test or diagnostic test for aneuploidy, including Down's syndrome (see Simpson and Elias[12] for a review). Several types of foetal cells can be recovered from maternal blood, such as trophoblasts, granuloctyes, and lymphocytes, of which the most successful have been nucleated foetal red cells. Given the rarity of these cells in maternal blood, about 1–2 foetal cells to 10 million maternal cells (although this may be higher in aneuploid foetuses), sophisticated techniques are required to obtain an adequate sample of foetal cells for analysis. Separation can be achieved using either flow sorting (fluorescent

TABLE 20 *Studies of foetal cells in maternal blood in the detection of Down's syndrome*

Study	Down's syndrome foetuses		Unaffected foetuses	
	Number	Percentage of cells in each sample with three signals. Range (median/mean)	Number	Percentage of cells in each sample with three signals. Range (median/mean)
Elias *et al*, 1992[15]	2	3, 74*	5	0–12 (7)
Ganshirt-Ahlert *et al*, 1994[16]	10	9–17 (12)	10	0–7 (2)
Simpson and Elias, 1993[17]	5	3–74 (19)	61	0 (0)
Valerio *et al*, 1993[18]	2	40, 70*	–	–

* *Actual values shown.*

activated cell sorting (FACS)) or magnetic activated cell sorting (MACS) techniques. Success is better with FACS, but it is a more complex technique than MACS and more expensive.[13,14]

The gestational age at which foetal cells can be most successfully retrieved needs to be determined, though it is suggested that cells can be adequately sampled between 10 and 18 weeks of pregnancy.[12] There is also a concern that foetal cells may persist in the maternal circulation from a previous pregnancy.

Once an adequate sample of foetal cells has been obtained techniques such as polymerase chain reaction or fluorescence *in situ* hybridisation can be used to identify an extra chromosome 21 (achieved by observing cells with three hybridisation 'signals'). Several studies have shown that the proportion of foetal cells which exhibit three signals is greater in Down's syndrome pregnancies than in unaffected pregnancies *(Table 20)*.

The method is in its early stages and a number of requirements need to be met before it can be of practical use[12,18,19] – namely, (a) adequate enrichment of foetal cells in the sample; (b) unequivocal distinction between foetal and maternal cells; (c) accurate methods for single cell diagnosis; and (d) acceptable cost. Further research is needed to determine if the technology will be useful in screening or diagnosis in a routine antenatal setting.

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Chapter 5

Demonstration projects

The value of demonstration projects lies in their ability to show the acceptability of screening and identify practical issues relating to implementation, such as the screening uptake rate. They are not designed to estimate screening performance and should not be used for this purpose; they lack statistical power and involve departures from screening protocols of indeterminate effect. It is, however, appropriate to examine whether the performance of screening in the demonstration projects is consistent with results expected from the primary studies. They provide a pragmatic indication of the effect of a particular screening programme.

Triple test demonstration projects

Table 21 shows the results of the ten demonstration projects using the triple test (AFP, uE_3, and total hCG). Variation in practice between screening programmes (for example, different risk cut-off values were used) means that detection rates and false-positive rates cannot readily be compared between projects.

The main results of the projects were as follows.

(a) Screening uptake – three of the studies reported screening uptake, with a mean uptake of 73%.

TABLE 21 *Triple test demonstration projects (maternal age with AFP, uE_3 and total hCG)*

Study (country)	Triple test offered to:	No. of women			Risk cut-off* 1 in:	Positive rate		Amnio-centesis uptake‡ (DS) (%)	DR (%)		Termination of DS pregnancy (%)¶
		Eligible	Screened	Uptake (%)		Initial (%)	After scan revision of gestation (%)		Observed	Estimated# at term	
Haddow *et al*, 1992 (USA)[1]	All	–	25,207	–	250	6.6	3.8	79 (20)	60 (21/35)	54	75 (15/20)
Phillips *et al*, 1992 (USA)[2]	< 35 years	–	9530	–	274†	7.2	3.2	70 (4)	57 (4/7)	51	100 (4/4)
Wald *et al*, 1992 (UK)[3]	All	≈17,000	12,603	74	250	5.7	4.1	77 (11)	48 (12/25)	42	90 (9/10)**
Cheng *et al*, 1993 (USA)[4]	All	–	7718	–	195†	8.0	6.0	69 (–)	91 (20/22)	89	–
Burton *et al*, 1993 (USA)[5]	All	–	8233	–	270†	10.4	5.9	81 (8)	83 (10/12)	79	–
Pescia *et al*, 1993 (Switzerland)[6]	All	–	7039	–	380	8.6	5.9	97 (11)	69 (11/16)	63	–
Piggott *et al*, 1994 (UK)[7]	All	10,443	6990	67	250	–	3.0	80 (8)	73 (8/11)	67	100 (8/8)
Goodburn *et al*, 1994 (UK)[8]	All	≈32,950	25,359	77	200	5.2	4.1	86 (33)	75 (36/48)	70	97 (32/33)
Mancini *et al*, 1994 (Italy)[9]	> 30 years	–	2892	–	380	–	13.3	– (3)	80 (4/5)	75	100 (3/3)
Kellner *et al*, 1995 (USA)[10]	≥ 35 years	–	10,605	–	270†	8.3	7.2	92 (11)	75 (12/16)	70	–
Mean of all		**–**	**–**	**73**	**–**	**7.5**	**5.7**	**81 (–)**	**70 (138/197)**	**64**	**91 (71/78)**

* *Women are screen positive if their risk of Down's syndrome (DS) at term exceeds the cut-off.*

† *Indicates that the risk at mid-trimester was used in the screening programme.*

‡ *The percentage of women with positive results who accepted an amniocentesis. The number of women with screen positive pregnancies with DS who accepted amniocentesis is indicated in brackets.*

\# *Adjusted for the natural foetal loss of DS pregnancies between the second trimester and term. If n = total number of DS observed and a = number of DS detected, then the DR (%) at term adjusted for foetal loss is 77a/(n–0.23a).*

¶ *The percentage of women with a DS pregnancy (diagnosed by amniocentesis) who chose to have a termination of pregnancy.*

** *One of the 11 women who underwent amniocentesis was lost to follow-up.*

(b) Screen positive rate – the mean initial screen positive rate was 7.5%, reducing to 5.7% after revision of gestational age using ultrasound scan among women with positive screening results. Screening programmes used different risk cut-off levels and they had different policies for the extent to which an ultrasound estimate of gestational age could differ from the dates gestational age before the ultrasound estimate was used to revise the test result (for example, the Bart's group[3] used a 17-day rule).

(c) Amniocentesis uptake – the mean uptake of amniocentesis after a screen positive result was 81%; the uptake of amniocentesis was, however, higher in affected pregnancies with positive results (92%, 109/118). This is likely to be due to the higher proportion of women with high risks, and such women are more likely to accept an amniocentesis.[1,3]

(d) Termination of pregnancy – six out of ten of the studies reported the uptake of termination of affected pregnancies,with a mean uptake of 91%.

(e) Detection rate – 70%. However, an estimated 23% of Down's syndrome pregnancies detected through screening in the second trimester will end in a foetal loss.[11,12] The pooled estimate of the live born term detection rate is therefore 64% (see footnote to *Table 21* for equation).

Double test demonstration projects

Table 22 shows the results of five demonstration projects using AFP and total hCG and two projects using AFP and free β-hCG.

TABLE 22 *Double test demonstration projects (maternal age with AFP and either total hCG or free β-hCG)*

Study (country)	Double test offered to:	No. of women			Risk cut-off* 1 in:	Positive rate		Amnio-centesis uptake† (DS) (%)	DR (%)		Termination of DS pregnancy (%)#
		Eligible	Screened	Uptake (%)		Initial (%)	After scan revision of gestation (%)		Observed	Estimated‡ at term	
AFP and total hCG											
Dawson *et al,* 1993 (UK)[13]	All	9283	8414	91	300	–	3.5	85 (7)	50 (7/14)	44	86 (6/7)
Burn, 1993 (UK)[14]	All	–	4898	–	–	3.5	3.5	78 (–)	57 (4/7)	51	–
Beekhuis, 1993 (The Netherlands)[15]	All	≈2282	2099	92	250	–	7.3	79 (4)	83 (5/6)	79	100 (3/3)**
Crossley *et al,* 1994 (UK)[16]	All	37,226	30,084	81	220¶	6.3	5.1	70 (21)	70 (26/37)	65	100 (21/21)
Mooney *et al,* 1994 (USA)[17]	All	–	12,170	–	307††	6.4	5.6	68 (10)	56 (10/18)	49	–
Mean		**–**	**–**	**88**	**–**	**5.4**	**4.9**	**76 (–)**	**63 (52/82)**	**57**	**97 (30/31)**
AFP and free β-hCG											
Spencer & Carpenter, 1993 (UK)[18]	All	9345	8317‡‡	89	300	7.1	5.3	89 (11)	69 (11/16)	63	91 (10/11)
Macri *et al,* 1994 (USA)[19]	< 35 years	–	44,272	–	365/380##	5.6	3.8	– (–)	69 (29/42)	63	–
Mean		**–**	**–**	**89**	**–**	**6.4**	**4.6**	**89 (–)**	**69 (40/58)**	**63**	**91 (10/11)**
Mean of all		**–**	**–**	**88**	**–**	**5.8**	**4.8**	**78 (–)**	**66 (92/140)**	**59**	**95 (40/42)**

DS = Down's syndrome.

* *Women are screen positive if their risk of DS at term exceeds the cut-off.*

† *The percentage of women with positive results who accepted an amniocentesis. The number of women with screen-positive pregnancies with DS who accepted amniocentesis is indicated in brackets.*

‡ *Adjusted for the natural foetal loss of DS pregnancies between the second trimester and term. If n = total number of DS observed and a = number of DS detected, then the DR (%) at term adjusted for foetal loss is 77a/(n–0.23a).*

The percentage of women with a DS pregnancy (diagnosed by amniocentesis) who chose to have a termination of pregnancy.

¶ *Indicates that the risk at mid-trimester was used in the screening programme.*

** *One of the four affected pregnancies diagnosed by amniocentesis resulted in a spontaneous foetal loss.*

†† *1 in 307 was the risk cut-off for women aged ≤ 36 years. Women > 36 years were screen positive if their risk based on age and serum markers exceeded the risk based on age alone.*

‡‡ *The results were based on 8179 singleton pregnancies after excluding 138 twin pregnancies.*

There were two hospitals in the study; one used a cut-off of 1 in 365, the other used a cut-off of 1 in 380.

The main results of the projects were as follows.

(a) Screening uptake – four of the studies reported screening uptake, with a mean uptake of 88%.
(b) Screen positive rate – the initial positive rate was 5.8%, reducing to 4.8% after revision of gestational age estimated by ultrasound among women with positive screening results.
(c) Amniocentesis uptake – all but one of the studies reported the amniocentesis uptake rate after a screen positive result. The mean uptake was 78%; the uptake in affected pregnancies was 90% (53/59), probably because they had higher risk values.
(d) Termination of pregnancy – four out of seven of the studies reported the uptake of termination of affected pregnancies with a mean uptake of 95%.
(e) Detection rate – the observed detection rate was 66% overall and 59% when adjusted for foetal loss. The detection rate for the double test with free β-hCG is somewhat higher than that of the double test using total hCG. There may, however, have been under ascertainment in the project by Macri and colleagues, 1994[19]; a total of 42 cases of Down's syndrome were seen in this population, but it has been estimated[20] from the age distribution of these women that 59 cases would have been expected, which would have reduced the estimated detection rate at term from 63% (29/42) to 49% (29/59). In any event, there are many variations in screening practice (such as the use of different risk cut-offs) at different centres which tend to invalidate a quantitative comparison of screening performance in different centres that use different markers.

Dried blood samples

It has been suggested that dried blood 'spots' on filter paper can be used instead of liquid serum or plasma in screening for Down's syndrome. Two groups have explored this development.[21,22] In general, this approach is likely to be more imprecise but it may still be sufficiently reliable for use in programmes in which samples have to be despatched through the post to the laboratory. The performance of screening using such collection methods has not been fully explored or quantified in comparison with the standard methods.

Conclusion

The demonstration projects confirm the feasibility and acceptability of serum screening conducted in different countries.Their results are also consistent with expected performance. They show that women in different countries tend to make similar decisions about accepting screening for a Down's syndrome pregnancy, accepting an amniocentesis, and having a termination of pregnancy, as observed by Haddow and Palomaki.[23] Uptake of screening was about 80% (average of 73% and 88%), uptake of amniocentesis in screen positive women was about 80%, (about 90% in affected pregnancies), and acceptance of a termination of pregnancy about 90%.

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Chapter 6

Ultrasound markers at 15–22 weeks of pregnancy

Two categories of ultrasound marker for Down's syndrome which are present after 14 weeks have been described. The first category comprises ultrasound markers, such as nuchal skin fold thickness, reduced femur or humerus length, pyelectasis (dilatation of renal pelvis) and hyperechogenic bowel. The other category comprises major foetal structural abnormalities that are associated with Down's syndrome – namely, congenital heart defects and duodenal atresia. The main reason for distinguishing these two categories of markers is that some clinicians feel that the presence of a major structural abnormality such as a heart defect is sufficient grounds for offering diagnostic amniocentesis or chorionic villus sampling (CVS), whereas the presence of an ultrasound marker, which is not, in itself, a severe abnormality, needs to be considered with other factors before offering an amniocentesis or CVS. The distinction is, however, not firm; some regard an increased nuchal fold thickness or even hyperechogenic bowel as sufficient indication for amniocentesis.

Table 23 gives summary estimates of the performance of nuchal fold thickness, femur and humerus length, pyelectasis, and hyperechogenic bowel as ultrasound markers for Down's syndrome in the second trimester of pregnancy from a review of the literature. Details of the relevant published studies are given in *Tables 24* to *32*;[2–44] only those studies in which both a detection rate and a false-positive rate were reported are presented. The summary estimates are based on a simple summation of affected and unaffected pregnancies from the studies combined and, because the quality of ultrasound has improved over recent years, studies published before 1989 and data obtained before 1987 have been excluded from this analysis. *Table 23* also shows the summary estimates from a literature review published by Vintzileos.[1] There are widely different estimates for both the detection and false-positive rates across the studies and considerable heterogeneity between them. The best ultrasound marker seems to be nuchal fold thickness, yielding a 38% detection rate for a 1.3% false-positive rate with estimates of the detection rate varying from 8% to 75% and estimates of the false-positive rate ranging from 0% to 8.5%. The other markers are substantially less discriminatory. *Table 33* summarises the studies on other less commonly

TABLE 23 *Summary estimates of screening performance derived from* Tables 24–31 *(excluding studies published before 1989 and data obtained before 1987) and compared with the review by Vintzileos*[1]

Ultrasound marker	Current review			Review by Vintzileos *et al*[1]		
	No. of studies	DR* (%)	FPR† (%)	No. of studies	DR (%)	FPR (%)
Nuchal fold thickness ≥ 6 mm	16	38	1.3	14	34	0.5
Femur length (comparing observed with expected)	10	34	5.9	10	29	8
Femur length (ratio of biparietal diameter to femur length)	4	22	5.9	8	37	6
Humerus length (comparing observed with expected)	6	37	5.3	3	31	5
Femur length and humerus length combined (comparing observed with expected)	3	36	3.7	2	32	2
Pyelectasis	4	19	2.4	3	21	3
Hyperechogenic bowel	3	11	0.7	2	9	1

* *Summary estimate for DRs derived by calculating total number of Down's syndrome pregnancies with positive results/total number of Down's syndrome pregnancies, and for FPRs derived by counting total number of unaffected pregnancies with positive results/total number of unaffected pregnancies.*

† *The FPRs exclude all pregnancies with an abnormal karyotype.*

described markers, including cerebral ventricular dilatation, choroid plexus cysts, ear length, fifth digit mid-phalanx hypoplasia, increased iliac length, and short frontal lobe.[18,22,45–48] None of these markers shows much promise.

Most of the studies reviewed were confined to high-risk pregnancies, such as women referred for prenatal diagnosis because of advanced maternal age or a positive serum screening test; studies which were confined to pregnancies in the general population were mainly for those markers where information on the marker is routinely obtained as part of the scan examination, such as femur length. Scanning is likely to be more carefully carried out in a high-risk population than in the general population because there is greater suspicion, leading to overestimates of the general population detection rate and false-positive rate in our summary of screening performance. However, because the best marker, nuchal fold thickness, seems to achieve a detection rate of 38%, this is the maximum detection rate expected if such screening were carried out routinely in the general population. This detection rate is half that of serum screening, and so rules out its use as a primary method of screening for Down's syndrome. Four studies which have examined the effectiveness of ultrasound screening for Down's syndrome in a low-risk population together identified only ten out of 57 (18%) affected pregnancies.[49–52]

The performance of second trimester ultrasound screening for Down's syndrome using the presence of structural abnormalities such as heart defects is uncertain. At birth, about 45–50% of infants with Down's syndrome have heart defects[53,54] (mainly atrioventricular canal defects or ventricular septal defects), whereas about 0.7% of all births have such a heart defect.[55] Both of these figures are likely to be higher for second trimester foetuses because some of these will die *in utero*. This gives the potential of ultrasound screening using heart defects as a marker to achieve a maximum detection rate of 45–50% of viable Down's syndrome pregnancies if almost all heart defects were detected by a routine second trimester ultrasound scan, but with a false-positive rate of at least 0.7%. Studies have shown a prevalence of chromosomal abnormalities in foetuses with a heart defect of 5–10%, and so a foetal karyotype is offered in such cases, regardless of the presence of other markers. About 4% of infants with Down's syndrome have duodenal atresia,[53] whereas fewer than 0.3% of all births have this abnormality.[56] Sonographic signs of duodenal atresia are often not apparent until after 24 weeks, and so ultrasound screening using this marker alone is unlikely to be effective.

The performance of screening could be improved by producing a risk estimate for Down's syndrome based on the presence or absence of a combination of ultrasound markers. However, information on the extent to which these markers are independent of each other in both affected and unaffected pregnancies would be needed. Similarly, if the correlation between the serum markers and ultrasound markers were known, the two methods of screening could be combined and, in theory, improve screening performance. This information is not available in the literature and would require a major research effort.

This review has shown that the maximum detection achievable with a single second trimester ultrasound marker is 38% using nuchal fold thickness or 45% using heart defects, both substantially less than the detection rate achievable with serum screening. Their use as a primary screening method for Down's syndrome is, therefore, ruled out. Despite the lack of clear evidence on the performance of second trimester ultrasound screening for Down's syndrome, it is now becoming common practice to perform such screening. A report from the National Down's Syndrome Cytogenetic Register showed that ultrasound was one of the indications for karyotyping in 23% of prenatal diagnoses of Down's syndrome between 1991 and 1992.[57] Such screening is carried out in two ways – either by seeking ultrasound markers at the routine anomaly scan at 18–20 weeks or by doing so in women with a positive serum screening result. The first is not only unsatisfactory for the reasons given above but, given the lack of information on the significance of some of the markers, clinicians are faced with difficult decisions on what action, if any, they should take. The second is also unsatisfactory because an ultrasound carried out after a positive serum screening result will, in the absence of ultrasound markers of Down's syndrome, tend to lower the estimate of risk, often changing a screen positive result into a screen negative result. Such a two-step procedure will systematically reduce detection and lead to a false-negative result in women who are told that they are at risk of having a Down's syndrome pregnancy through having a positive serum screening test. It is a policy that should be resisted.

We do not dismiss the potential value of an anomaly scan, but it should not be used alone as a primary screening test for Down's syndrome and should not be used as a secondary test in women with positive serum screening results.

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(References continued on page 46)

TABLE 24 *Nuchal skin fold thickness: DRs and FPRs in screening for Down's syndrome in the second trimester, using a cut-off of 6 mm (screen positive = nuchal thickness ≥ 6 mm)*

Study	Gestation (weeks)	No. of Down's syndrome	No. of controls	DR (%)	FPR (%)	Type of population	Period of study
Benacerraf *et al,* 1987[2]	15–20	21	3804	43	0.1	High risk	1983–87
Perrella *et al,* 1988[3]	15–21	14	128	21	9.4	High risk	1981–87
Benacerraf *et al,* 1989[4]	15–20	20	3500	40	0.3	High risk	1987–89
Lynch *et al,* 1989[5]	18–22	9	9	56	0	High risk	1985–88
Ginsberg *et al,* 1990[6]	14–20	12	212	42	0	High risk	Not reported
Nyberg *et al,* 1990[7]	16–20	25	3500	16	0.3	High risk	1984–90
Benacerraf *et al,* 1991[8]	14–20	24	400	50	0	High risk	1988–89*
Crane & Gray, 1991[9]	14–21	16	3322	75	1.1	General	1988–90
Benacerraf *et al,* 1992[10]	14–20	32	588	69	0.3	High risk	1990–91
Kirk *et al,* 1992[11]	15–20	19	7094	47	0.3	General	1988–90
de Vore & Alfi, 1993[12]	14–23	35	2752	20	0.5	High risk	1990–93
Lockwood *et al,* 1993[13]	13–22	42	4949	14	0.6	High risk	1989–92
Benacerraf *et al,* 1994[14]	14–21	45	106	36	0	High risk	1991–93
Donnenfeld *et al,* 1994[15]	14–20	13	1346	8	1.2	High risk	1991–92
Gray & Crane, 1994[16]	14–18	26	6301	35	0.2	General	1988–92
Gray & Crane, 1994[16]	19–24	6	1805	83	3.5	General	1988–92
Watson *et al,* 1994[17]	14–21	14	1381	50	2.0	High risk	Not reported
Bahado-Singh *et al,* 1995[18]	14–21	8	640	50	1.4	High risk	1992–94
de Vore & Alfi, 1995[19]	17 (mean)	17	1000	12	0.8	High risk	1990–92
Grandjean *et al,* 1995[20]	14–24	44	3205	39	8.5	High risk	1989–92
Borrell *et al,* 1997[21]	14–18	29	1421	38	0.1	High risk	1991–93

* *Assumed.*

TABLE 25 *Nuchal skin fold thickness: DRs and FPRs in screening for Down's syndrome in the second trimester, using a cut-off of 5 mm (screen positive = nuchal thickness ≥ 5 mm)*

Study	Gestation (weeks)	No. of Down's syndrome	No. of controls	DR (%)	FPR (%)	Type of population	Period of study
Toi *et al,* 1987[22]	15–20	11	28	18	21.0	High risk	Not reported
Lockwood *et al,* 1993[13]	13–22	42	4949	29	3.7	High risk	1988–92
Gray & Crane, 1994[16]	14–18	26	6301	42	2.8	General	1988–92
Gray & Crane, 1994[16]	19–24	6	1805	100	18.7	General	1988–92
Nyberg *et al,* 1995[23]	15–18	18	232	17	0.4	High risk	1990–91

TABLE 26 *Femur length measurement (observed compared with expected femur length): DRs and FPRs in screening for Down's syndrome in the second trimester*

Study	No. of Down's syndrome	No. of controls	DR (%)	FPR (%)	Definition of screen positive*	Type of population	Period of study
Benacerraf *et al,* 1987[24]	28	192	68	2	O/E ≤ 0.91	High risk	1983–87
Perrella *et al,* 1988[3]	19	128	26	5	O/E ≤ 0.91	High risk	1981–87
Benacerraf *et al,* 1989[4]	20	709	40	5	O/E ≤ 0.91	High risk	1987–89
Cuckle *et al,* 1989[25]	83	1340	24	6.3	O/E ≤ 0.90	General	1986–89
Dicke *et al,* 1989[26]	33	177	15	10	O/E < 0.90	General	1981–88
Hill *et al,* 1989[27]	22	286	50	15	O/E ≤ 0.91	General	1985–88
LaFollette *et al,* 1989[28]	30	229	13	12	O/E ≤ 0.91	General	1986–87
Lynch *et al,* 1989[5]	9	9	56	56	O/E ≤ 0.91	High risk	1985–88
Peters *et al,* 1989[29]	16	194	13	7	O/E ≤ 0.91	High risk	1985–88
Grist *et al,* 1990[30]	6	428	50	9.8	O/E ≤ 0.91	High risk	1985–87
Nyberg *et al,* 1990[31]	49	572	14	6.0	O/E ≤ 0.91	High risk	1983–88
Benacerraf *et al,* 1991[8]	24	400	42	10	O/E ≤ 0.91	High risk	1988–89†
Rodis *et al,* 1991[32]	11	1890	18	5	O < 5th percentile	General	1988–90
Biagiotti *et al,* 1992[33]	16	1163	44	8.6	O/E ≤ 0.91	General	1987–90
Rotmensch *et al,* 1992[34]	43	204	19	9	O/E ≤ 0.90	High risk	1985–90
Lockwood *et al,* 1993[13]	41	4874	15	3	O–E < –3.4 mm	High risk	1989–92
Nyberg *et al,* 1993[35]	45	942	24	4.7	O/E ≤ 0.91	High risk	1990–91
Benacerraf *et al,* 1994[14]	45	106	44	4.0	O/E ≤ 0.91	High risk	1991–93
Biagiotti *et al,* 1994[36]	27	500	48	12	O/E ≤ 0.91	High risk	1987–92
Johnson *et al,* 1995[37]	36	794	42	16	O/E(ga) ≤ 0.90	High risk	Not reported
Nyberg *et al,* 1995[23]	18	232	28	6.0	O/E ≤ 0.91	High risk	1990–91

**O = observed femur length; E = expected femur length, as estimated by biparietal diameter; E(ga) = expected femur length, as estimated by last menstrual period. † Assumed.*

TABLE 27 *Femur length measurement (ratio of biparietal diameter to femur length): DRs and FPRs in screening for Down's syndrome in the second trimester*

Study	No. of Down's syndrome	No. of controls	DR (%)	FPR (%)	Definition of screen positive*	Type of population	Period of study
Lockwood *et al,* 1987[38]	55	544	58	6	B/F > 1.5 sd	High risk	1984–86
Brumfield *et al,* 1989[39]	15	45	40	2	B/F ≥ 1.8	High risk	1983–87
Dicke *et al,* 1989[26]	33	177	18	4	B/F > 1.5 sd	General	1981–88
Hill *et al,* 1989[27]	22	286	36	7	B/F > 1.5 sd	General	1985–88
Lynch *et al,* 1989[5]	9	9	22	11	B/F > 1.5 sd	High risk	1985–88
Peters *et al,* 1989[29]	16	194	12	7	B/F > 1.5 sd	High risk	1985–88
Ginsberg *et al,* 1990[6]	11	212	46	7	B/F > 1.5 sd	High risk	Not reported
Marquette *et al,* 1990[40]	31	155	10	9	B/F > 1.5 sd	High risk	Not reported
Shah *et al,* 1990[41]	17	17	18	6	B/F > 90th percentile	High risk	1983–87
Rodis *et al,* 1991[32]	11	1470	18	5	B/F > 95th percentile	General	1988–90
Johnson *et al,* 1995[37]	26	542	27	7	B/F ≥ 1.8	High risk	Not reported

** B = biparietal diameter measurement; F = femur length measurement; sd = standard deviation.*

TABLE 28 *Humerus length measurement: DRs and FPRs in screening for Down's syndrome in the second trimester*

Study	No. of Down's syndrome	No. of controls	DR (%)	FPR (%)	Definition of screen positive*	Type of population	Period of study
Benacerraf *et al,* 1991[8]	24	400	50	6	O/E < 0.90	High risk	1988–89†
Rodis *et al,* 1991[32]	11	1890	64	5	O < 5th percentile	General	1988–90
Rotmensch *et al,* 1992[34]	43	204	28	9	O/E < 0.90	High risk	1985–90
Lockwood *et al,* 1993[13]	21	2775	29	4	O–E < –3.6 mm	High risk	1989–92
Nyberg *et al,* 1993[35]	45	942	24	5	O/E ≤ 0.89	High risk	1990–91
Biagiotti *et al,* 1994[36]	27	500	56	15	O/E ≤ 0.91	High risk	1987–92
Johnson *et al,* 1995[37]	33	486	24	4	O/E ≤ 0.90	High risk	Not reported

** O = observed humerus length; E = expected humerus length, as estimated by biparietal diameter. † Assumed.*

TABLE 29 *Humerus and femur length measurements combined: DRs and FPRs in screening for Down's syndrome in the second trimester*

Study	No. of Down's syndrome	No. of controls	DR (%)	FPR (%)	Definition of screen positive*	Type of population	Period of study
Benacerraf *et al,* 1992[10]	32	588	53	3.9	$O^f/E^f \leq 0.91$ and $O^h/E^h < 0.90$	High risk	1990–91
Rotmensch *et al,* 1992[34]	43	204	16	6	O^f/E^f and $O^h/E^h < 0.90$	High risk	1985–90
Nyberg *et al,* 1993[35]	45	942	18	1.6	$O^f/E^f \leq 0.91$ and $O^h/E^h \leq 0.89$	High risk	1990–91
Biagiotti *et al,* 1994[36]	27	500	44	7.6	O^f/E^f and $O^h/E^h \leq 0.91$	High risk	1987–92

* *O^f =observed femur length; O^h = observed humerus length; E^f = expected femur length, as estimated by biparietal diameter; E^h = expected humerus length, as estimated by biparietal diameter.*

TABLE 30 *Foot length combined with humerus and femur length measurements: DRs and FPRs in screening for Down's syndrome in the second trimester*

Study*	No. of Down's syndrome	No. of controls	DR (%)	FPR (%)	Definition of screen positive†	Type of population
Johnson *et al,* 1995[37]	27	501	41	8	O^h/foot length ≤ 0.85	High risk
Johnson *et al,* 1995[37]	30	741	57	13	O^f/foot length ≤ 0.90	High risk
Johnson *et al,* 1995[37]	27	495	52	7	$(O^f + O^h)$/foot length ≤ 1.75	High risk

* *Period of study not reported.*
† *O^h = observed humerus length; O^f = observed femur length.*

TABLE 31 *Pyelectasis: DRs and FPRs in screening for Down's syndrome in the second trimester*

Study	No. of Down's syndrome	No. of controls	DR (%)	FPR (%)	Type of population	Period of study
Benacerraf *et al,* 1990[42]	44	7400	25	2.8	General	1988–89
Corteville *et al,* 1992[43]	23	5876	17	2.0	General	1988–90
de Vore & Alfi, 1995[19*]	17	1000	6	1.6	High risk	1990–92
Nyberg *et al,* 1995[23]	18	232	17	2.2	High risk	1990–91

* *Using colour Doppler® ultrasound.*

TABLE 32 *Hyperechogenic bowel: DRs and FPRs in screening for Down's syndrome in the second trimester*

Study	No. of Down's syndrome	No. of controls	DR (%)	FPR (%)	Type of population	Period of study
Bromley *et al,* 1994[44]	48	8680	12	0.6	General	1991–93
de Vore & Alfi, 1995[19*]	17	1000	12	1.2	High risk	1990–92
Nyberg *et al,* 1995[23]	18	232	6	2.2	High risk	1990–91

* *Using colour Doppler® ultrasound.*

TABLE 33 *Ultrasound markers other than those in* Tables 24–32: *DRs and FPRs in screening for Down's syndrome in the second trimester*

Ultrasound marker	Study	No. of Down's syndrome	No. of controls	DR (%)	FPR (%)	Type of population	Period of study
Cerebral ventricular dilatation	Nyberg *et al*, 1995[23]	18	232	6	0	High risk	1990–91
Choroid plexus cyst	de Vore & Alfi, 1995[19]	17	1000	0	1.8	High risk	1990–92
Ear length	Lettieri *et al*, 1993[45]	9	410	78	8.0	High risk	1991
Fifth digit mid-phalanx hypoplasia	Benacerraf *et al*, 1990[46]	8	1024	75	18	High risk	1987–88
Increased iliac length	Abuhamad *et al*, 1994[47]	10	180	50	2	High risk	1992–93
Short frontal lobe	Bahado-Singh *et al*, 1992[48]	19	125	21	4.8	High risk	Not reported

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Chapter 7

Serum and ultrasound screening at 10–14 weeks of pregnancy

Serum markers

All the serum markers used or considered in the second trimester have been assessed in Down's syndrome and unaffected pregnancies during the first trimester.[1–49] *Figures 12* to *18* and *Table 34* show a review of the literature of serum markers for Down's syndrome at 10–14 weeks of pregnancy, presented in the same way as for the second trimester markers (chapter 3). *Table 35* shows the detection rate for a 5% false-positive rate for each serum marker with maternal age. Two serum markers stand out as being useful in screening at 10–14 weeks – namely, PAPP-A and free β-hCG. A third, uE_3, may also be useful (see *Figure 15*). The addition of the other markers

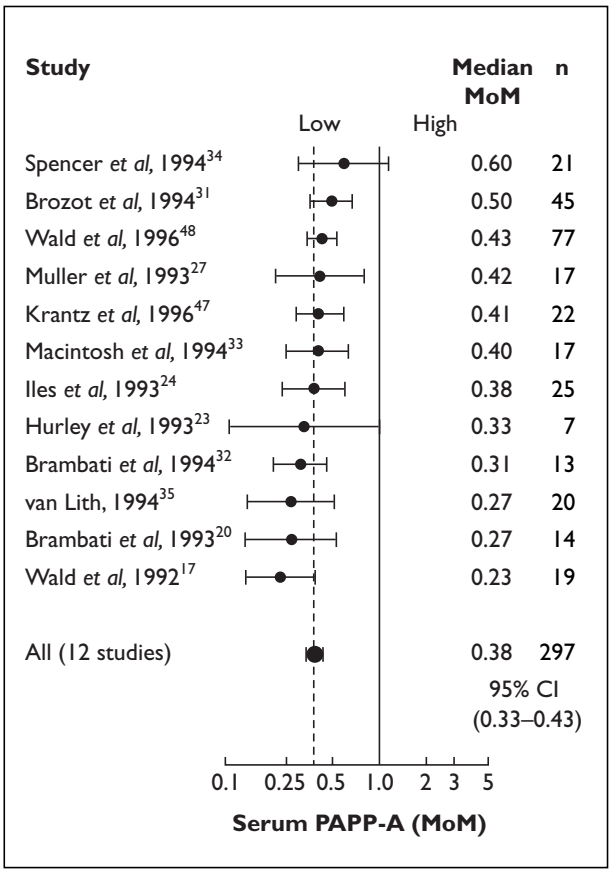

FIGURE 12 *Median PAPP-A (MoM) at 10–14 weeks in Down's syndrome pregnancies, with 95% CI and the number of affected pregnancies in each study (the mean value was used when the median could not be obtained). The pooled median is indicated by the vertical broken line*

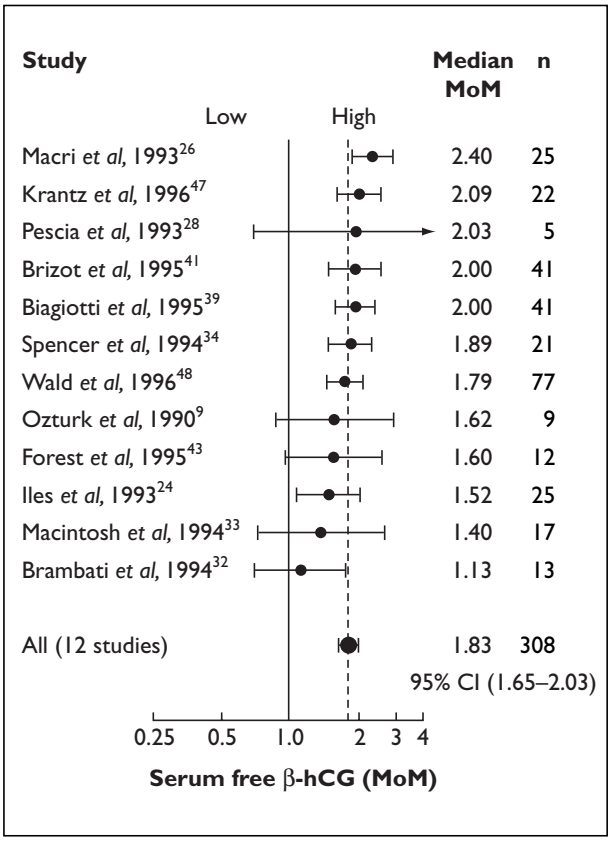

FIGURE 13 *Median free β-hCG (MoM) at 10–14 weeks in Down's syndrome pregnancies, with 95% CI and the number of affected pregnancies in each study (the mean value was used when the median value could not be obtained). The pooled median is indicated by the vertical broken line*

increases the detection rate by a further 1–2% only. *Figure 19* shows the distribution of risk based on PAPP-A and free β-hCG combined with maternal age in affected and unaffected pregnancies at 10–14 weeks. *Table 36* summarises screening performance using maternal age and these two markers.[50] At a risk cut-off level of 1:300, the screening detection rate is 63% for a 5.5% false-positive rate with an odds of being affected given a positive result of 1:67. This screening performance is comparable with second trimester screening using the triple test but less effective than that using the quadruple test. It is also possible that the markers are associated with spontaneous foetal loss, and this would decrease the performance of screening when judged in terms of live-born term pregnancies.

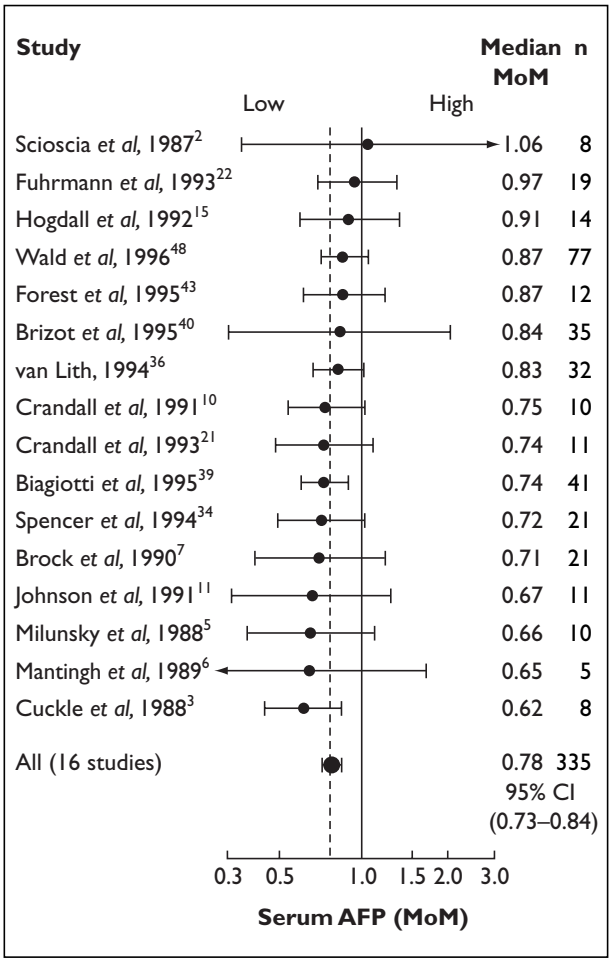

FIGURE 14 *Median AFP (MoM) at 10–14 weeks in Down's syndrome pregnancies, with 95% CI and the number of affected pregnancies in each study (the mean value was used when the median value could not be obtained). The pooled median is indicated by the vertical broken line*

Figure 20 combines first and second trimester results and shows how the median MoM values in Down's syndrome pregnancies change with gestational age (using data from the FiTSS study[48] and the Oxford–Bart's data).[51,52,54] Important results emerge from the figure:

(a) PAPP-A loses discrimination after 13 weeks, also corroborated by other work (see *Table 2*, chapter 3, and Cuckle, 1994 (*Table 23.6*)[55])
(b) free α-hCG is low in affected pregnancies before about 12 weeks and high thereafter
(c) inhibin A is relatively non-discriminatory before 14 weeks.

Nuchal translucency measurement

The ultrasound marker of choice before 15 weeks of pregnancy is nuchal translucency measurement. *Figure 21* illustrates this measurement. The reviews

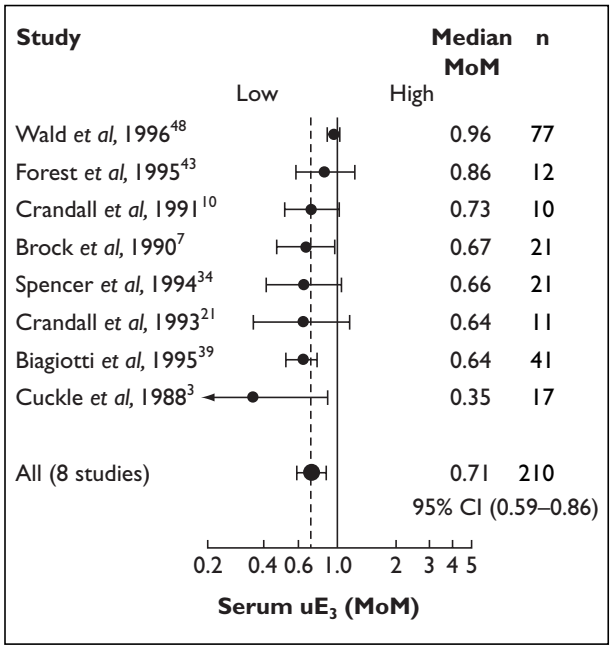

FIGURE 15 *Median uE_3 (MoM) at 10–14 weeks in Down's syndrome pregnancies, with 95% CI and the number of affected pregnancies in each study (the mean value was used when the median value could not be obtained). The pooled median is indicated by the vertical broken line*

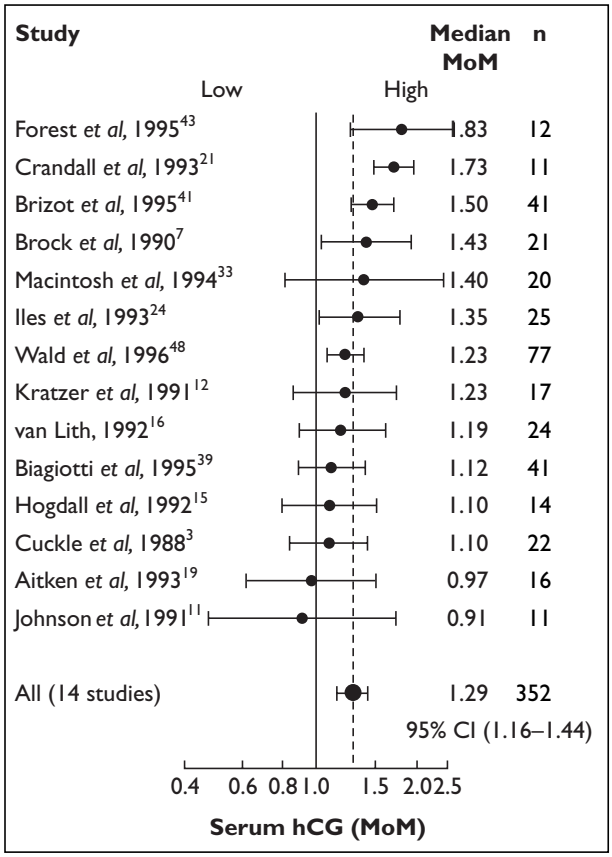

FIGURE 16 *Median hCG (MoM) at 10–14 weeks in Down's syndrome pregnancies, with 95% CI and the number of affected pregnancies in each study (the mean value was used when the median value could not be obtained). The pooled median is indicated by the vertical broken line*

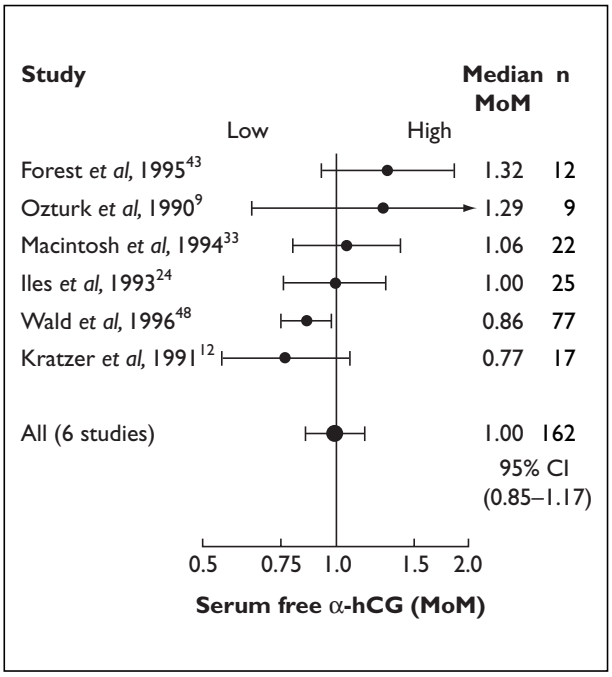

FIGURE 17 *Median free α-hCG (MoM) at 10–14 weeks in Down's syndrome pregnancies, with 95% CI and the number of affected pregnancies in each study (the mean value was used when the median value could not be obtained). The pooled median is indicated by the vertical broken line*

by Snijders and Nicolaides[56] and by Kornman and colleagues[57] together list 23[57–79] studies on first trimester foetal nuchal translucency in relation to Down's syndrome. Fifteen of these studies[58–61,64–70,72,74,75,79] were restricted to women who had foetuses with an increased nuchal translucency measurement or a cystic hygroma. This permits the estimation of the positive predictive value (which will depend on the prevalence of Down's syndrome in the population screened as well as the performance of the test) but not the estimation of the detection rate and false-positive rate. The remaining eight studies plus six additional studies found in the literature[57,62,63,71,73,76–78,80–85] provide the necessary information to estimate detection and false-positive rates for nuchal translucency in relation to Down's syndrome; *Table 37* summarises these studies. They can be divided into two groups:

(a) those that relate to women who had an ultrasound scan before an amniocentesis or CVS on account of a high risk of a foetal abnormality, usually advanced maternal age ('high-risk' women)
(b) those in whom nuchal translucency measurement was conducted routinely in a general antenatal population ('routine' screening).

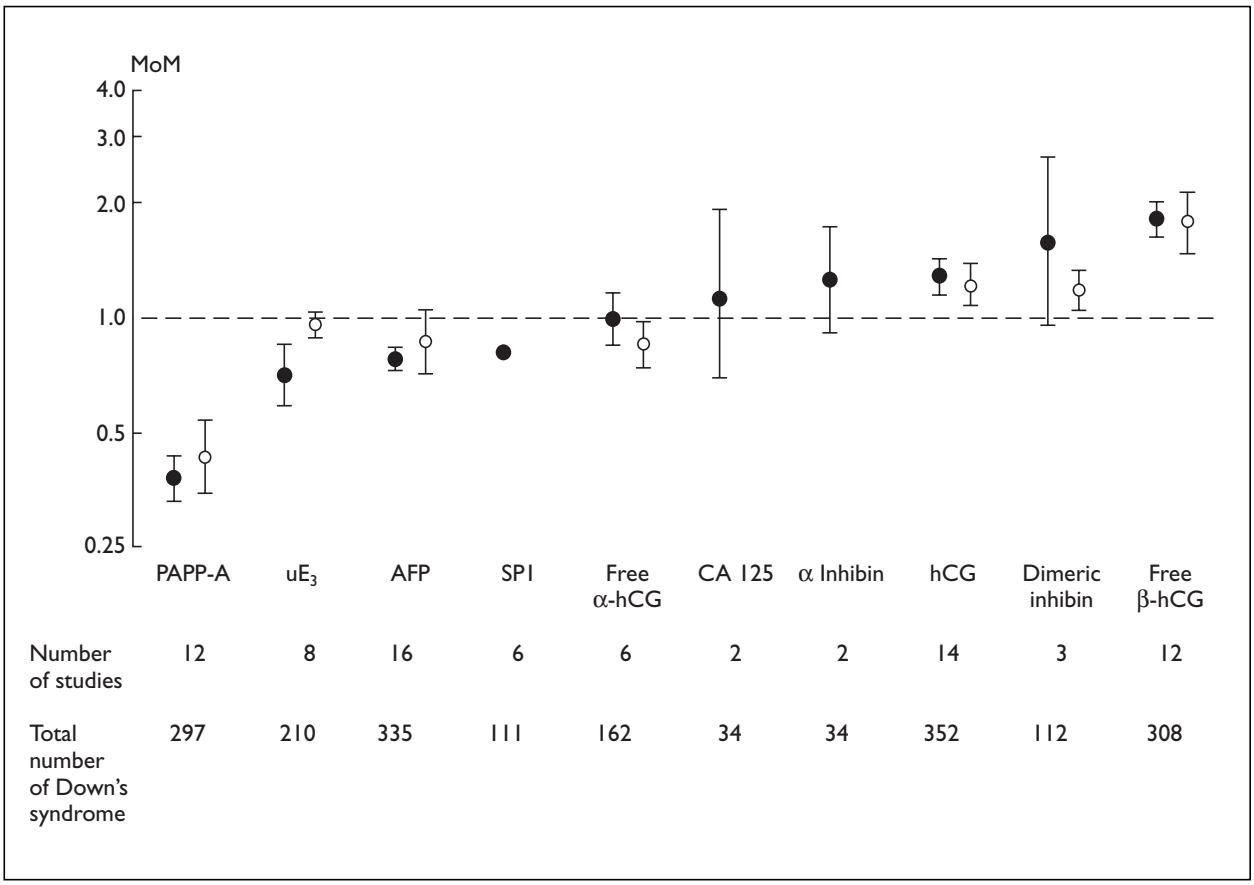

FIGURE 18 *The pooled median and 95% CI (solid circle) at 10–14 weeks of pregnancy from studies shown in* Figures 12 *to* 17 *compared with the estimate from the Oxford–Bart's data set (open circle)*[10]

TABLE 34 *Median MoM (and 95% CI) at 10–14 weeks of pregnancy for inhibin, SP1 and CA 125 in Down's syndrome pregnancies*

Serum marker and study	No. of Down's syndrome	Median MoM	95% CI
α ***Inhibin***			
van Lith *et al,* 1994[37]	23	1.25	(0.66–2.37)
Wallace *et al,* 1994[38]	11	1.30	(0.67–2.49)
All (2 studies)	**34**	**1.27**	**(0.92–1.75)**
Dimeric inhibin A			
Wallace *et al,* 1995[45]	23	2.46	(1.84–3.29)
Aitken *et al,* 1996[46]	14	1.38	(0.82–2.32)
Wald *et al,* 1996[48]	75	1.19	(1.05–1.35)
All (3 studies)	**112**	**1.59**	**(0.96–2.65)**
CA 125			
Hogdall *et al,* 1992[15]	14	1.60	(0.69–3.70)
van Lith *et al,* 1993[29]	20	0.97	(0.84–1.11)
All (2 studies)	**34**	**1.14**	**(0.72–1.81)**
SP1			
Brock *et al,* 1990[7]	19	0.79	
Aitken *et al,* 1993[18]	14	0.73	
Macintosh *et al,* 1993[25]	14	0.40	(0.21–1.77)
Pescia *et al,* 1993[28]	5	1.29	
Brizot *et al,* 1995[42]	45	0.96	
Qin *et al,* 1997[49]	14	0.89	(0.20–2.09)
All (6 studies)*	**111**	**0.81**	

** The pooled median was obtained as the average of the median MoM (in logs) in each study weighted by the number of Down's syndrome pregnancies, as the standard deviation was only available for two studies.*

TABLE 35 *Down's syndrome screening performance of serum markers at 8–14 weeks' gestation*

Maternal age with:	DR (%) for a 5% FPR
PAPP-A	52
Free β-hCG	38
AFP	32
Total hCG	32
Free α-hCG	32
Dimeric inhibin A	31
uE_3	30
Free β-hCG + PAPP-A	62

Source: Wald et al, *1995.*[50]

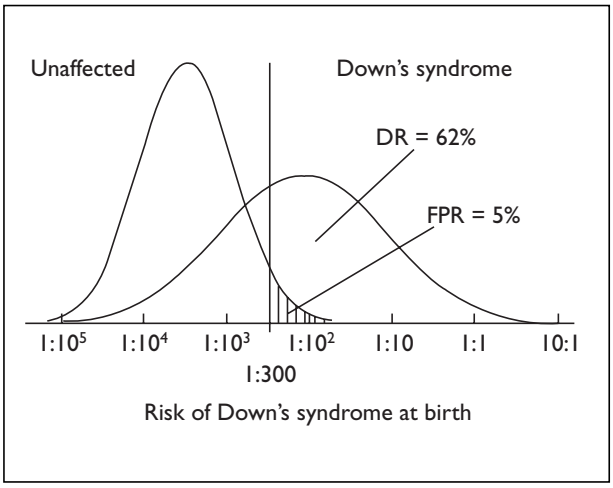

FIGURE 19 *The distribution of risk in unaffected and Down's syndrome pregnancies using maternal age with PAPP-A and free β-hCG at 10–14 weeks of pregnancy*

TABLE 36 *Down's syndrome screening at 8–14 weeks' gestation using maternal age with free β-hCG + PAPP-A*

Risk cut-off level	DR (%)	FPR (%)	OAPR
1:100	45	1.5	1:25
1:200	57	3.5	1:47
1:300	63	5.5	1:67
1:400	68	7.4	1:84
1:500	72	9.5	1:102

Source: Wald et al, *1995.*[50]

Two conclusions can be drawn from these data. Firstly, the test is highly discriminatory. Secondly, the point estimates from different studies are statistically inconsistent with each other, suggesting that there are sources of variation that can influence the results in an unpredictable manner. The studies reviewed in *Table 37* used a fixed nuchal translucency measurement cut-off level, without taking account of gestational age or maternal age. Some of the heterogeneity between studies on nuchal translucency measurement could be due to differences in gestational age because nuchal translucency measurement increases with increasing gestational age between 10 and 13 weeks of pregnancy. On average, nuchal translucency over this period increases by an estimated 17% for each week of gestation.[88]

Screening performance will be improved by adjusting nuchal translucency measurement for gestational age. Nicolaides and colleagues[86] did

this by expressing nuchal translucency measurements as deviations from the expected nuchal translucency measurement at a given crown rump length, using crown rump length as an indication of gestational age. *Table 38* shows their results using this approach together with maternal age for routinely screened women and for 'high-risk' women (that is, those referred for CVS). The reported detection rate in routine screening was 84% for a false-positive rate of 5.8% using a risk cut-off of 1 in 300 (risk calculated on the basis of maternal age and nuchal thickness adjusted for gestational age). This estimate, however, is an overestimate because of the method of calculating the detection rate.[87] Cases of Down's syndrome detected in the first trimester (n = 36) were used in the numerator and cases detected plus cases missed used in the denominator (n = 43). About 48% of the cases detected would have resulted in a spontaneous foetal loss,[89,90] so the estimate of the term detection rate is $(36 \times 52\%)/((36 \times 52\%) + 7)$ – that is, $19/(19 + 7)$, yielding a detection rate of 73% (95% CI, 56–90) instead of 84%. This estimate may still be too high if increased nuchal translucency measurement among Down's syndrome pregnancies is associated with spontaneous foetal loss, for which there is a suggestion in both affected and unaffected pregnancies.[91,92]

Ultrasound in the first trimester has been increasingly used in recent years to identify pregnancies at risk of Down's syndrome. In 1989, four affected pregnancies were detected in this way in Britain and, in 1995, 107 were detected *(Figure 22)*.

In summary, ultrasound screening in the first trimester of pregnancy is effective and is being used more widely but its screening performance has not been reliably specified. The following issues need to be addressed.

1. There is heterogeneity between the published estimates of screening performance, so one cannot be sure that results in one centre can be replicated in another. This heterogeneity needs to be explained and understood, so that results from centres with good screening performance can be transported to others.

2. On physical grounds, the resolution of a single ultrasound image cannot be greater than the wavelength of the sound wave used, and the effective resolution will be at least twice as large as this. For a 3 MHz transducer the wavelength of sound is 0.5 mm, and the effective image resolution is at least 1 mm. With higher frequency transducers the resolution will be

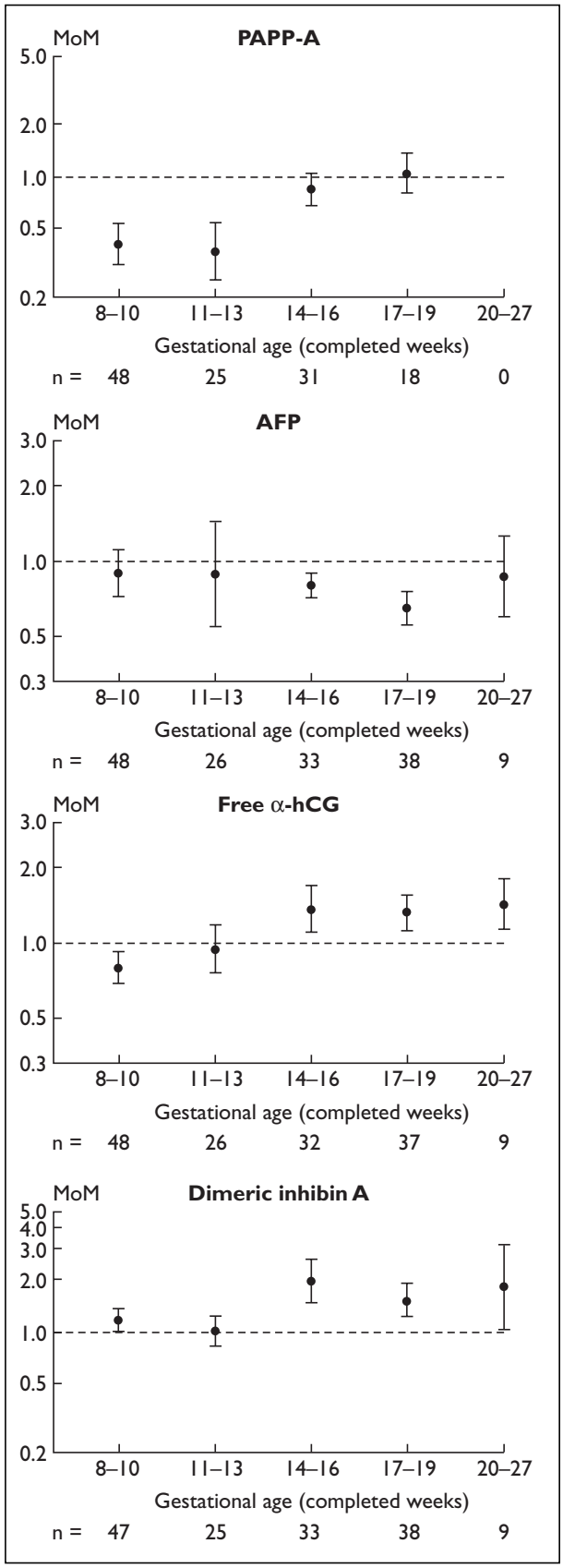

FIGURE 20 *Median serum marker levels (MoM) and 95% CI in Down's syndrome pregnancies according to gestational age from 8 to 27 weeks[48,51,52,54] (with additional data from Knight* et al, *1993[53] for PAPP-A from 16 weeks of pregnancy)* *continued*

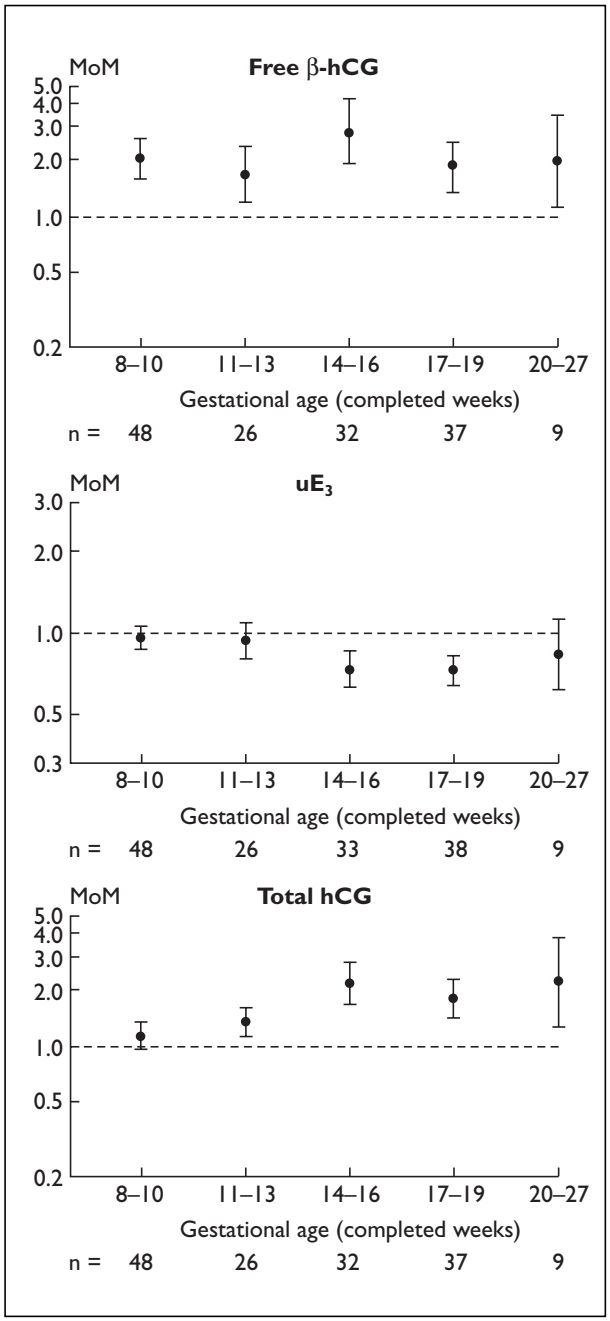

FIGURE 20 contd *Median serum marker levels (MoM) and 95% CI in Down's syndrome pregnancies according to gestational age from 8 to 27 weeks[48,51,52,54] (with additional data from Knight* et al, *1993[53] for PAPP-A from 16 weeks of pregnancy)*

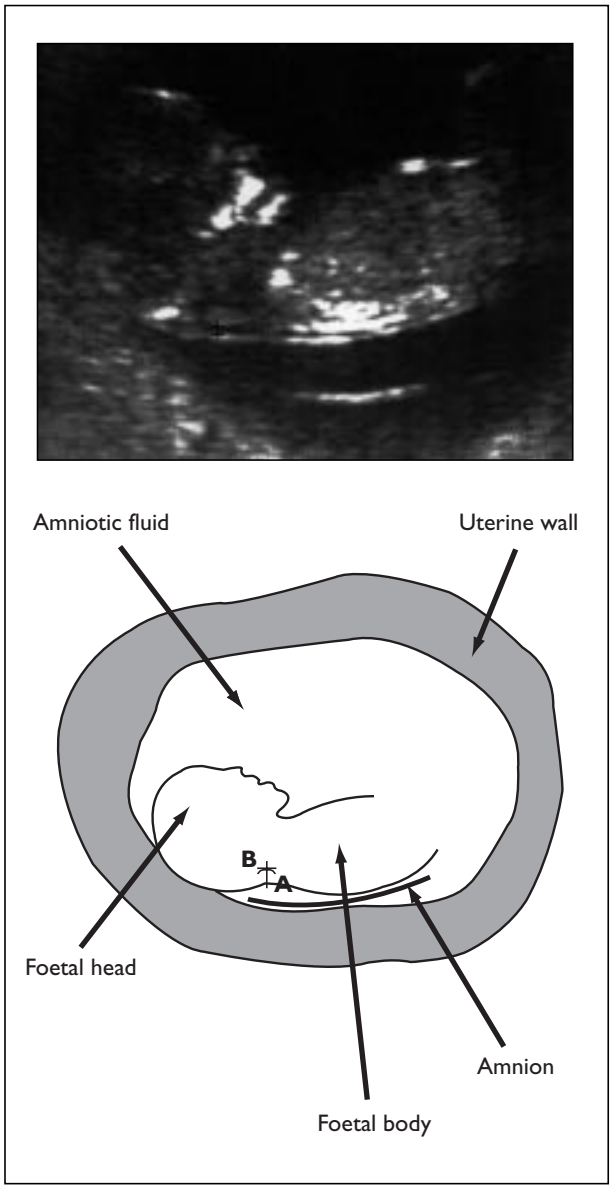

FIGURE 21 *Illustration of nuchal translucency measurement at 10–14 weeks of pregnancy (A – B = nuchal translucency)*

higher; a 5 MHz transducer will have an effective image resolution of about 0.4 mm and for a 6 MHz transducer it will be about 0.3 mm; however, there are disadvantages in using high-frequency transducers, and so they are not currently widely used. Because nuchal translucency measurements lie between about 0 and 5 mm, with an overlap between affected and unaffected pregnancies around 2–3 mm, it follows that measurement imprecision will have a practical influence on screening performance. It will make a relatively large contribution to the variance of the measurement in both affected and unaffected pregnancies, so increasing the overlap of the two distributions and thereby increasing the false-positive rate while reducing the detection rate at a given cut-off level. There is, therefore, a paradox between the reports of high screening performance from some centres, and the physical limitations of the method. An explanation needs to be found. There must be specific techniques used by those centres that are obtaining good results that have not been explicitly recorded. For example, those centres may take repeated measurements

TABLE 37 *Nuchal translucency measurement as a screening test for Down's syndrome in the first trimester in studies which specified both DR and FPR; women are screen positive if nuchal translucency is ≥ 3 mm*

Study	Transducer (MHz)	Abdominal/ vaginal ultrasound	Gestation weeks (median)	Reported DR (%)	Reported FPR* (%)
Women referred for amniocentesis of CVS					
Szabo *et al,* 1990[62]	6.5	Vaginal	11–12	100 (7/7)	1.0 (1/105)
Nicolaides *et al,* 1994[73]	5	Abdominal	10–13 (11)	84 (21/25)	4.5 (55/1227)
Brambati *et al,* 1995[77]	3.5/5	Both	8–15 (10)	27 (7/26)	3.2 (57/1776)‡
Comas *et al,* 1995[78]	5	Vaginal	9–13 (11)	57 (4/7)	9.3 (42/453)
Szabo *et al,* 1995[83]	6.5	Vaginal	9–12	89 (24/27)	2.8 (35/1243)
Kornman *et al,* 1996[57]	3.5/5	Abdominal	≤ 13	0 (0/1)	5.0 (13/260)
Borrell *et al,* 1997[85]	3.5	Abdominal	10–13 (11)	44 (8/18)	6.3 (29/462)
All (7 studies)#	**–**	**–**	**–**	**64 (71/111)**	**4.2 (232/5526)**
Women routinely screened¶					
Bower *et al,* 1995[80]	3.5/5	Abdominal	Not specified	45 (5/11)	6.1 (159/2624)
Bewley *et al,* 1995[76]	6.5	Vaginal	8–14 (11)	33 (1/3)	6.1 (68/1122)
Szabo *et al,* 1995[83]	–	–	9–12	100 (4/4)	0.9 (18/2091)
Kornman *et al,* 1996[57]	3.5/5	Abdominal	≤ 13	67 (2/3)	7.8 (21/270)
All (4 studies)	**–**	**–**	**–**	**57 (12/21)**	**4.4 (266/6107)**

* *Excluding pregnancies with chromosomal abnormalities.*
† *Two of the studies also gave results for nuchal translucency ≥ 4 mm: Comas* et al, *1995;[78] DR = 57% (4/7), FPR = 0.7% (3/453); and Nicolaides* et al, *1994;[73] DR = 60% (15/25), FPR = 1.0% (12/1227). Two additional studies only gave results for ≥ 4 mm: Schulte-Vallentin & Schindker, 1992;[63] DR = 100% (7/7), FPR = 0.2% (1/625); and Savoldelli* et al, *1993;[71] DR = 54% (15/28), FPR = 0.4% (5/1357).*
‡ *Corrected figures (Brambati B, personal communication).*
\# *Excluding Haddow & Palomaki, 1996[84] (DR = 53%) because of the very high FPR (30%).*
¶ *Two additional studies only gave results for nuchal translucency ≥ 2.5 mm: Pandya* et al, *1995;[82] DR = 75% (3/4), FPR = 3.4% (59/1758) and Hafner* et al, *1995;[81] DR = 50% (2/4), FPR = 0.8% (16/1959).*

TABLE 38 *Reported screening performance* for nuchal translucency measurement (adjusted for gestational age) with maternal age*

	Reported DR (%)	Reported FPR (%)
Women routinely screened	84 (36/43)	5.8 (1280/22,033)
Women referred for CVS (that is, 'high risk')	88 (91/104)	15.7 (3211/20,439)

* *Estimates of screening performance are overestimated (see text and Hackshaw* et al, *1996[87]).*
Source: Nicolaides et al, *1996.[86]*

and, although these may not be recorded, the operator may mentally choose an average value for his report. Such an informal 'averaging' approach could have a large effect on increasing precision and might explain the results. Whatever the explanation, the distribution of nuchal translucency in affected and unaffected pregnancies and the underlying components of variation need to be quantified. This will help specify a standard technique that can be adopted at all centres to ensure a predictable level of screening performance.

3. There is uncertainty over the proportion of pregnancies in which a nuchal translucency measurement is obtainable. Three studies[73,81,82] achieved a 100% success rate in all pregnancies in which a measurement was attempted. Two studies,[84,92] however, were unable to obtain a measurement in 18% of pregnancies. This has

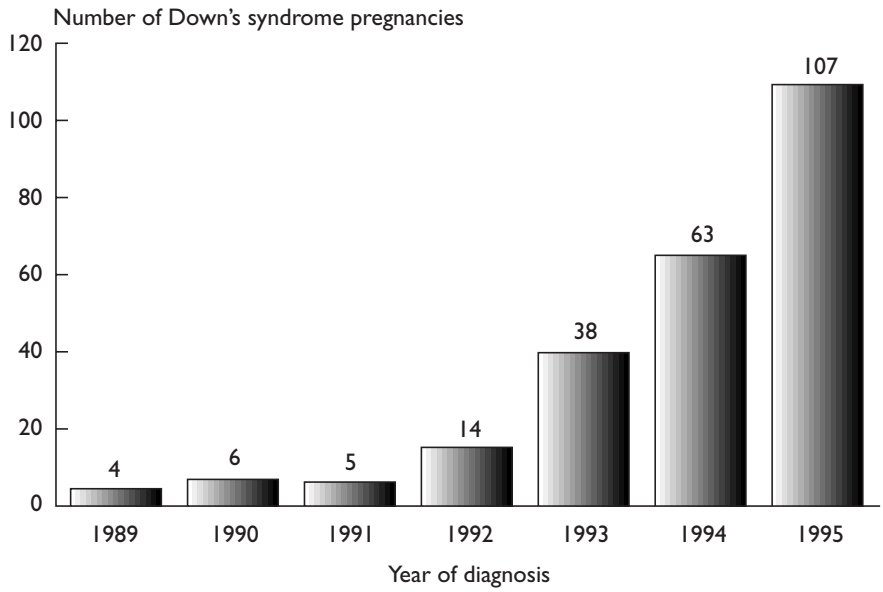

FIGURE 22 *The number of Down's syndrome pregnancies detected by ultrasound before 15 weeks of pregnancy in Britain between 1989 and 1995 (National Down's Syndrome Cytogenetic Register)*

important implications for routine screening and needs to be addressed. One study[92] showed that the failure rate was highest at the early (8 weeks) and later (13 weeks) part of the first trimester, and there were also difficulties caused by an unsuitable foetal position and maternal obesity. The failure rate decreases if more time is spent on the examination of each woman and if women are recalled after an unsuccessful attempt. This will, however, incur extra costs, and can disorganise the work of busy antenatal clinics.

4. A screening algorithm and method of statistical analysis has only recently been made explicit and published in sufficient detail for others to calculate risk estimates.[96] The parameters that define the distribution of nuchal translucency in affected and unaffected pregnancies have also recently been specified, but they are still based on assumptions and need to be clarified through further research.

5. There is uncertainty over the precision of estimating the nuchal translucency thickness. This can be considered in terms of within-person variation and between-person variation.

(i) Within-person variation

One study[92] showed that when the same sonographer measured the same foetus on two occasions (on each occasion the foetus was measured twice and the sonographer was blind to his/her first result) 31% of 86 nuchal thickness measurements (in 43 foetuses) differed by 1 mm or more. Two studies[93] (and unpublished data from Schuchter K, Austria) found the variation to be less; Pandya and colleagues[82] estimated that for 95% of foetuses (n = 200), two measurements on the same foetus would differ by not more than ± 0.54 mm, whereas the other study (from Austria), in which six measurements were taken on each of 561 foetuses, estimated that the difference between the smallest and largest measurement would not be more than ± 0.46 mm for 95% of foetuses. Taking repeated measurements is of value because it will lead to a reduction in the standard deviation of nuchal translucency measurements: in the study from Austria, the standard deviation for nuchal translucency ($\log_{10}$ MoM) was 0.17 using a single measurement and 0.14 using the mean of six measurements. The effect on screening performance would be an increase in the detection rate for a given false-positive rate.

(ii) Between-person variation

The three studies mentioned above also examined the effect of two sonographers measuring nuchal translucency on the same foetus. One study[92] reported that the nuchal thickness differed by more than 1 mm in 71% of foetuses, a relatively large between-person variation. The two other studies estimated that for 95% of foetuses,the difference would

not be more than ± 0.62 mm (n = 200)[93] or ± 0.54 mm (n = 133),[88] similar to the within-person variation.

Both of these considerations have implications for sonographer training, risk estimation, and screening practice. Important factors are likely to include time spent on each woman, quality and specifications of the ultrasound machine, and sonographer experience.

6. Screening performance may vary according to gestational age and this has not been fully described. A general method needs to be adopted to take account of the increase in nuchal translucency with gestational age – for example, by using multiples of the normal median.

7. As mentioned above, it is possible that an increased nuchal translucency is associated with a higher miscarriage rate.[91,92] A non-interventional study is needed, in which nuchal translucency is measured and recorded with full ascertainment of the outcomes of pregnancy to investigate this reliably.

8. Any screening programme based on nuchal translucency measurement would require specification of the quality of the ultrasound marker, sonographer experience, procedure and quality control, and a realistic assessment of the duration of each examination with a policy for how to deal with failure to obtain a measurement.

Combining serum markers and nuchal translucency measurement

Once the performance of ultrasound screening using nuchal translucency measurement has been reliably specified and the technique has been shown to be reproducible and transportable to any centre, it will have to be combined with serum screening in the first trimester (using free β-hCG and PAPP-A). This will require determining the correlation between the different markers in both affected and unaffected pregnancies to assess the extent to which the markers are independent measures of the risk of a Down's syndrome pregnancy. Data on this suggest that PAPP-A, AFP, total hCG, and free β-hCG are independent of nuchal translucency.[31,40,41,94]

Research is also needed to examine the extent to which serum markers and nuchal translucency measurement are associated with spontaneous foetal loss in affected and unaffected pregnancies, for which there is a suggestion in the literature.[91,92,95]

Termination of affected pregnancies

Termination of a foetal abnormality is a distressing experience at any time in pregnancy. A termination early in pregnancy is likely to be less distressing than one performed later, provided that the care and support offered with an early termination is as good as that provided for a later termination. The method of termination before 13 weeks (suction curettage) is often regarded as less traumatic than methods used later (prostaglandin induction), but consideration needs to be given to the fact that about one-quarter of pregnancies with Down's syndrome miscarry between 10 and 15 weeks of pregnancy.[89,90] Therefore, women with an early antenatal diagnosis of an affected pregnancy will be offered a termination of pregnancy when they have a one in four chance that the pregnancy would otherwise abort spontaneously over the next few weeks. There is no published information about how women view the difference between spontaneous and induced abortion in the presence of an abnormality, and whether one is more distressing than the other.

Current position

A policy for first trimester nuchal translucency screening for Down's syndrome must be compared with the existing method of screening. At present, second trimester screening can achieve a detection rate of 76% for a 5% false-positive rate. A preliminary assessment of first trimester screening using nuchal translucency, free β-hCG, and PAPP-A measurement with maternal age yielded an estimated detection rate of 80% for a 5% false-positive rate, but this needs corroboration.[96] Preliminary evidence suggests, therefore, that differences in performance between first and second trimester screening may be small, even after the uncertainty associated with earlier screening is resolved. The final assessment should be determined mainly on efficacy, safety, and cost. The potential change in policy arising from moving from second to first trimester screening will be important, with implications for training, staffing, and antenatal practice. Another consideration is that the added benefit of AFP screening for NTDs would be lost. Our conclusion is that first and second trimester screening should be fully evaluated quantitatively before decisions are reached on altering current screening practice.

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Chapter 8

Methods of antenatal diagnosis

An acceptably safe and effective method of antenatal diagnosis must be available if antenatal screening is to be a practical proposition. Screening before 9 weeks of pregnancy is not appropriate because subsequent diagnosis by CVS carries a risk of causing limb reduction defects if performed earlier than 10 weeks.[1]

In this chapter we examine the evidence on safety and efficacy of the two methods of antenatal diagnosis – amniocentesis and CVS. To avoid bias, we used the results of the randomised trials of amniocentesis (before and after 13 weeks of pregnancy) and CVS (transcervical and transabdominal methods) usually performed between 9 and 13 weeks of pregnancy. The trials are examined for:

(a) foetal loss
(b) failure to obtain a sample
(c) uninformative results due to culture failure, placental mosaicism or maternal cell contamination.

There are seven randomised trials on women referred for antenatal diagnosis, mainly because of advanced maternal age,[2–9] and one trial[10] which studied women aged 25–34 years and was the only one to have a control group of women who did not have an invasive procedure. *Tables 39* to *41* show background details of the randomised trials.

The eight studies used different criteria for the inclusion of study subjects in the statistical analysis, and different criteria for estimating safety and efficacy. For example, some studies excluded foetal losses that occurred after randomisation but before the diagnostic procedure was attempted, while some studies included these foetal losses. Also, some studies were analysed according to the allocated diagnostic procedure and some according to the diagnostic procedure that was undertaken in practice. Such differences make it difficult to combine the study results and, for consistency, the estimates of safety and efficacy were recalculated from the raw data given in the papers using the

TABLE 39 *Second trimester amniocentesis and first trimester CVS: details of randomised trials*

Description of trial	Scan for viability before randomisation	Lost to follow-up (%)	% in whom assigned procedure was not performed			
			Amniocentesis 13–20 weeks	TC-CVS 9–13 weeks	TA-CVS 9–13 weeks	No invasive procedure
Amniocentesis vs. no invasive procedure						
Danish (Tabor *et al*);[10] 4672 randomised ≤ 19 weeks	No	0.1	1.7	–	–	1
TC-CVS vs. amniocentesis						
European;[2] 3234 randomised at 9 weeks (mean)*	Yes	1.0	7.9 (no invasive procedure) 2.7 (CVS)	5.0 (no invasive procedure) 1.9 (amniocentesis)	–	–
Canadian;[3] 2787 randomised at < 12 weeks†	No	2.3	32	26	–	–
Danish (Smidt-Jensen *et al*);[4,5] 3079 randomised at < 11weeks‡	Yes	Not reported	–	–	–	–
TA-CVS vs. amniocentesis						
Danish (Smidt-Jensen *et al*);[4,5] 3079 randomised at < 11 weeks†	Yes	Not reported	–	–	–	–

TA-CVS = transabdominal CVS; TC-CVS = transcervical CVS.

* *72% had TC–CVS; 28% had TA–CVS; excludes multiple pregnancies.*

† *Analysis excluded women found to have non-viable foetus after randomisation.*

‡ *2.7% did not have assigned procedure and authors excluded these data from analyses; included only women randomised after withdrawal of the use of a metal cannula; randomised for TA-CVS also.*

TABLE 40 *First trimester amniocentesis vs. TA-CVS: details of randomised trials*

Trial	Number randomised	Week randomised	Scan for viability before randomised	Lost to follow-up (%)	% in whom assigned procedure was not performed	
					Early TA-CVS at 9–13 weeks	Amniocentesis at 10–13 weeks
King's College, London[6]	488	10–13	Yes	0	0	0

TABLE 41 *First trimester TA-CVS vs. TC-CVS: details of randomised trials*

Trial	Number randomised	Week randomised	Scan for viability before randomised	Lost to follow-up (%)	% in whom assigned procedure was not performed	
					TC-CVS at 9–13 weeks	TA-CVS at 9–13 weeks
Bologna[7]	120	9–13	Yes	0	0	0
Milan[8]	1194	7–12	Yes	0.9	18.4	6.4
Philadelphia[9]	3999	7–12	Yes	0.4	3.3 (no invasive procedure) 3.3 (TA-CVS)	3.1 (no invasive procedure) 3.6 (TC-CVS)
Danish (Smidt-Jensen *et al*)[5]	2927	< 11	Yes	Not reported	6.3 (TA-CVS)	0.6 (TC-CVS) 0.1 (amniocentesis)

following standard criteria. (If the data were unavailable for analysis in this way, this is indicated in the comments column given in the relevant table.)

Foetal loss rate

- Foetal losses were taken to be any losses after randomisation (that is, including all spontaneous losses, terminations, stillbirths, and neonatal deaths). This method will overestimate the absolute risk of procedure-induced foetal losses but avoids making assumptions about the miscarriage rate of foetuses with an abnormality; the difference in foetal loss rate between the randomised groups is an unbiased estimate of the excess associated with the more hazardous procedure.
- In the control group of the Danish trial (Tabor *et al*)[10] the number of infants born alive with a chromosomal abnormality or NTD was added to the foetal losses because affected pregnancies in the study group were detected and terminated.
- All women randomised were included and an 'intention-to-treat' analysis used; sufficient published data were, in general, available for this.
- Women 'lost to follow-up' were excluded from the analyses.

Rate of failure to obtain a sample

- Sample failure was taken as obtaining insufficient sample for a technically satisfactory karyotype at the first visit, with no more than two attempts at the procedure allowed.
- Only women for whom a procedure was attempted were included.
- The results were analysed according to the allocated procedure.
- Women 'lost to follow-up' were excluded from the analyses.

Rate of uninformative results

- Uninformative results were taken to occur whenever a reliable foetal karyotype was not obtained owing to a culture failure, placental mosaicism, or maternal cell contamination.
- Only women for whom a procedure was attempted were included.
- The results were analysed according to the allocated procedure.

- Women 'lost to follow-up' were excluded from the analyses.

In addition to our analysis of the studies, details of the safety and efficacy of the different procedures given in the trials and a comparison with the results from the systematic review by Neilson in the Cochrane Pregnancy and Childbirth Database are presented at the end of this chapter in *Tables 45* to *54*.[11]

Safety

The following conclusions on the safety of the different methods of antenatal diagnosis emerge from *Table 42*.

1. The foetal loss rate due to amniocentesis is 0.9% (95% CI, 0.0–1.9) (Danish trial, Tabor *et al*[10]; *Table 42*) with a mid-range estimate (25–75th percentile) of 0.6–1.2%.
2. Transcervical CVS seems to result in more foetal losses than mid-trimester amniocentesis and possibly first trimester transabdominal CVS, but the issue is not totally resolved. Combining results of three trials on transcervical CVS and amniocentesis showed that transcervical CVS has a statistically significant excess risk of foetal loss of 3.7%, compared with amniocentesis (*Table 42*). Combining the results of four trials on transcervical CVS and transabdominal CVS showed that transcervical CVS has an excess risk of foetal loss of only 1.1% compared with transabdominal CVS, but this difference was not statistically significant *(Table 42)*.
3. Transabdominal CVS and mid-trimester amniocentesis each have a similar excess risk of foetal loss. This is supported by direct evidence from the Danish trial reported by Smidt-Jensen and colleagues[5] (*Table 42)*.
4. Early amniocentesis is associated with a higher risk of foetal loss than transabdominal CVS; the excess risk of foetal loss was 3.6% (95% CI, –0.8–8; *Table 42)*.

Statistical analyses reported by the Cochrane Centre yield similar results. Reasons for the differences are given in the comments columns of *Tables 45* to *48*.

In summary, mid-trimester amniocentesis and transabdominal CVS after about 10 weeks of

TABLE 42 *Relative foetal loss rates for different methods of antenatal diagnosis*

Trial	Difference in foetal loss rate				
	Amniocentesis vs. no invasive procedure (%)	**TC-CVS vs. amniocentesis (%)**	**TA-CVS vs. amniocentesis (%)**	**TC-CVS vs. TA-CVS (%)**	**Early amniocentesis vs. TA-CVS (%)**
Danish (Tabor *et al*)[10]	0.9				
European[2]		4.6			
Canadian[3]		1.7			
Danish (Smidt-Jensen *et al*)[5]		4.5	–0.1	4.5	
Bologna[7]				0	
Milan[8]				–0.9	
USA[9]				–0.2	
King's College, London[6]					3.6
Combined (95% CI)	**0.9 (0.0–1.9)**	**3.7 (2.0–5.5)**	**–0.1 (–2.2–2.0)**	**1.1 (–1.9–4.2)**	**3.6 (–0.8–8.0)**

Figures derived from Tables 45–48.

TABLE 43 *Rate of failure to obtain a sample from different methods of antenatal diagnosis*

Trial	Rate of failure to obtain a sample			
	Amniocentesis (%)	**TC-CVS (%)**	**TA-CVS (%)**	**Early amniocentesis (%)**
Danish (Tabor *et al*)[10]	0.0			
European[2]	1.7	5.1		
Canadian[3]	2.1	9.5		
Danish (Smidt-Jensen *et al*)[5]	0.0	4.2	1.7	
Bologna[7]		23	13	
Milan[8]		0.2	0.2	
USA[9]		2.5	1.4	
Danish (Smidt-Jensen *et al*)[5]		3.1	1.2	
King's College, London[6]			0.7	0.0
Combined (95% CI)	**0.8 (0.5–1.0)**	**4.3 (3.8–4.8)**	**1.3 (1.0–1.6)**	**0.0 (0.0–1.5)**

Details of trials are in Tables 49–51.

TABLE 44 *Rate of uninformative results* from different methods of antenatal diagnosis*

Trial	Rate of uninformative results			
	Amniocentesis (%)	**TC-CVS (%)**	**TA-CVS (%)**	**Early amniocentesis (%)**
Danish (Tabor *et al*)[10]	1.0			
European[2]	0.7	1.4		
Canadian[3]	0.1	2.3		
Danish (Smidt-Jensen *et al*)[5]	0.5	1.3	1.4	
Bologna[7]		0.0	0.0	
Milan[8]		1.5	0.7	
USA[9]		< 1	< 1	
Danish (Smidt-Jensen *et al*)[5]		1.0	0.6	
King's College, London[6]			1.8	2.5
Combined (95% CI)	**0.6 (0.4–0.8)**	**1.5 (1.2–1.8)**	**1.0 (0.7–1.3)**	**2.5 (1.3–3.6)**

* *Due to culture failure, placental mosaicism, and maternal cell contamination.*
Details of trials are in Tables 52–54.

pregnancy are of comparable safety, with an estimated foetal loss rate of just under 1%. The studies reviewed covered varying periods, the earliest being the Danish (Tabor *et al*) trial,[10] which studied pregnancies undergoing amniocentesis between 1982 and 1984.

Levels of experience among clinicians performing the procedures also varied between the studies. Improvements in technique and equipment over time may therefore mean that efficacy and safety have improved; it is widely perceived by clinicians, for example, that the foetal loss rate following second trimester amniocentesis is lower than 0.9%. The lower 95% CI is 0% so a lower risk is quite possible. Without full and unbiased data, uncertainty will remain. When comparing different methods of screening (see chapter 9, *Table 57*), the absolute foetal loss rate due to amniocentesis is not critical; the choice of screening method can still be made because the relative advantages of different screening methods are unaltered by changes in the absolute rate. However, estimates of the overall efficacy and safety for a particular method of screening may be uncertain. In current practice, it is probably reasonable to say that the true foetal loss rate is unlikely to be greater than about 1.5% and likely to be less than 0.9%.

Efficacy

Tables 43 and *44* provide the following conclusions on the efficacy of different methods of antenatal diagnosis. The most effective method of antenatal diagnosis is mid-trimester amniocentesis. This is more likely to yield a sample for examination and is more likely to be informative. If the results from all the studies are combined, a sample was not obtained in 0.8% (95% CI, 0.5–1.0) of amniocentesis procedures. The rate for transcervical CVS was 4.3% (95% CI, 3.8–4.8) and for transabdominal CVS 1.3% (95% CI, 1.0–1.6).

The summary estimate of the proportion of uninformative results obtained after an amniocentesis was 0.6% (95% CI, 0.4–0.8). The rate was 1.5% (95% CI, 1.2–1.8) for transcervical CVS and 1.0% (95% CI, 0.7–1.3) for transabdominal CVS. In summary, mid-trimester amniocentesis is the most effective diagnostic procedure. As discussed in relation to our estimates of foetal loss rates, the studies reviewed involved procedures performed as early as 1982 so we may have overestimated the proportion of amniocenteses for which no sample was obtained (0.8%). Even if the true estimate is lower than this, it is unlikely to alter practice.

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TABLE 45 *Foetal loss rates of second trimester amniocentesis vs. no invasive procedure*

Trial	Foetal loss after randomisation						Main difference between this analysis and Cochrane analysis	Comments on this analysis
	Amnio-centesis (%)	No invasive procedure (%)	Difference* (95% CI) (%)	Mid-range estimate of difference (i.e. 50% CI) (%)	Odds ratio† (95% CI)	Odds ratio from Cochrane analysis (95% CI)		
Amniocentesis vs. no invasive procedure								
Danish (Tabor *et al*)[10]	3.2 (73/2302)	2.3 (52/2304)	0.9 (0.0–1.9)	(0.6–1.2)	1.4	3.2 (1.7–5.9)	Cochrane includes only women pregnant at the time of amniocentesis (study group) or pregnant at the end of the 16th week (control group)	Includes all women randomised

* *Rate of foetal loss in the amniocentesis group minus rate in group with no invasive procedure.*
† *Ratio of odds of foetal loss between amniocentesis group and group with no invasive procedure.*

TABLE 46 *Foetal loss rates of first trimester TC-CVS vs. second trimester amniocentesis, and first trimester TA-CVS vs. second trimester amniocentesis*

Trial	Foetal loss after randomisation							Main difference between this analysis and Cochrane analysis	Comments on this analysis
	Amnio-centesis (%)	TC-CVS (%)	TA-CVS (%)	Difference* (95% CI) (%)	Mid-range estimate of difference (i.e. 50% CI) (%)	Odds ratio† (95% CI)	Odds ratio from Cochrane analysis (95% CI)		
TC-CVS vs. amniocentesis									
European[2]	9.0 (144/1592)	13.7 (220/1609)	–	4.6 (2.4–6.8)	(3.9–5.4)	1.6 (1.3–2.0)	NA	Cochrane analyses data for TC-CVS and TA-CVS separately using unpublished data	All data analysed as TC-CVS, though 28% had TA-CVS
Canadian[3]	15.2 (207/1361)	16.9 (230/1363)	–	1.7 (–1.1–4.4)	(0.7–2.6)	1.1 (0.9–1.4)	1.1 (0.9–1.4)	Cochrane includes women 'lost to follow-up' in denominators	
Danish (Smidt-Jensen *et al*)[5]	6.4 (67/1042)	10.9 (110/1010)	–	4.5 (2.0–6.9)	(3.6–5.3)	1.8 (1.3–2.4)	1.7 (1.3–2.2)	Cochrane includes all women randomised regardless of procedure used	Restricted to one period where method of TC-CVS unchanged
Combined	**NA**	**NA**	**NA**	**3.7 (2.0–5.5)**	**(3.1–4.3)**	**1.5 (1.1–1.9)**	**1.3 (1.1–1.5)**	**Excludes MRC trial**	
TA-CVS vs. amniocentesis									
Danish (Smidt-Jensen *et al*)[5]	6.4 (67/1042)	6.3 (65/1027)	–	–0.1 (–2.2–2.0)	(–0.8–0.6)	1.0 (0.8–1.2)	1.1 (0.8–1.5)	See Danish TC-CVS above	

* *Rate of foetal loss in relevant CVS group minus rate in amniocentesis group.*
† *Ratio of odds of foetal loss between relevant CVS group and amniocentesis group.*
NA = not available.

TABLE 47 *Foetal loss rate of first trimester TA-CVS vs. early* amniocentesis*

Trial	Foetal loss after randomisation					
	Early amniocentesis (%)	**TA-CVS (%)**	**Difference† (95% CI) (%)**	**Mid-range estimate of difference (i.e. 50% CI) (%)**	**Odds ratio‡ (95% CI)**	**Odds ratio from Cochrane analysis (95% CI)**
King's College, London[6]	8.4 (20/238)	4.8 (12/250)	3.6 (–0.8–8.0)	(2.1–5.1)	1.8 (0.9–3.8)	1.8 (0.9–3.7)

* *Before 13 weeks.*
† *Rate of foetal loss in early amniocentesis group minus rate in TA-CVS group.*
‡ *Ratio of odds of foetal loss between early amniocentesis and TA-CVS groups.*

TABLE 48 *Foetal loss rates of first trimester TA-CVS vs. TC-CVS*

Trial	Foetal loss after randomisation						Main difference between this analysis and Cochrane analysis	Comments on this analysis
	TC-CVS (%)	**TA-CVS (%)**	**Difference* (95% CI) (%)**	**Mid-range estimate of difference (i.e. 50% CI) (%)**	**Odds ratio† (95% CI)**	**Odds ratio from Cochrane analysis (95% CI)**		
Bologna[7]	3.3 (2/60)	3.3 (2/60)	0 (–6.4–6.4)	(–2.2–2.2)	1.0 (0.1–16.4)	1.0 (0.1–7.3)	–	–
Milan[8]	15.7 (93/592)	16.6 (98/591)	–0.9 (–5.1–3.3)	(–2.3–0.6)	0.9 (0.7–1.3)	1.1 (0.7–1.7)	Cochrane excludes foetuses found to be non-viable after randomisation and includes women lost to follow-up	–
Philadelphia[9]	7.8 (157/2001)	8.1 (160/1978)	–0.2 (–1.9–1.4)	(–0.8–0.3)	1.0 (0.8–1.2)	0.9 (0.6–1.3)	Cochrane excludes abnormal foetal losses and includes neonatal deaths	–
Danish (Smidt-Jensen *et al*)[5]	12.0 (175/1457)	7.6 (111/1470)	4.5 (2.3–6.6)	(3.7–5.2)	1.7 (1.3–2.1)	2.7 (1.9–3.9)	Cochrane includes only women randomised three ways – excludes two-way analysis	Includes women randomised two ways and three ways (see *Table 41*)
Combined	**NA**	**NA**	**1.1 (–1.9–4.2)**	**(0.1–2.2)**	**1.1 (0.8–1.6)**	**1.4 (1.3–1.5)**	**Cochrane includes abstract by Tomassini[12] (44 women only)**	**–**

* *Rate of foetal loss in TC-CVS group minus rate in TA-CVS group.*
† *Ratio of odds of foetal loss between TC-CVS and TA-CVS groups.*
NA = not available.

TABLE 49 *Failure to obtain a sample from second trimester amniocentesis compared with CVS*

Trial	Failure to obtain a sample						Main difference between this analysis and Cochrane analysis	Comments on this analysis
	Amnio-centesis (%)	TC-CVS (%)	TA-CVS (%)	Difference* (95% CI) (%)	Odds ratio† (95% CI)	Odds ratio from Cochrane analysis (99% CI)		
TC-CVS vs. amniocentesis								
European[2]	1.7 (25/1467)	5.1 (78/1528)	–	3.4 (2.1–4.7)	3.1 (2.0–4.9)	2.9 (1.7–4.8)	Cochrane included all women randomised whether or not a procedure was carried out	Included only women where a procedure was carried out
Canadian[3]	2.1 (18/861)	9.5 (95/998)	–	7.4 (5.4–9.5)	4.9 (3.0–8.2)	Not reported	–	Up to three aspirations allowed and at least 10 mg of tissue obtained
Danish (Smidt-Jensen *et al*)[4]	0.0 (0/961)	4.2 (38/900)	–	4.2 (2.9–5.5)	Incalculable	Not reported	–	Figures taken from 1991 paper[4] – total number of procedures slightly lower than 1992 paper;[5] reason unknown
Combined				**4.9 (2.8–6.9)**	**4.0 (2.2–7.2)**	**Not reported**	**–**	**–**
TA-CVS vs. amniocentesis								
Danish (Smidt-Jensen *et al*)[4]	0.0 (0/961)		1.7 (17/1018)	1.7 (0.9–2.5)	Incalculable	Not reported	–	Figures taken from 1991 paper[4] – total number of procedures slightly lower than 1992 paper;[5] reason unknown

* *Rate of sample failure in relevant CVS group minus rate in amniocentesis group.*

† *Ratio of odds of sample failure between relevant CVS group and amniocentesis group.*

TABLE 50 *Failure to obtain a sample after TA-CVS compared with early amniocentesis*[*]

Trial	Failure to obtain a sample					Main difference between this analysis and Cochrane analysis	Comments on this analysis
	Early amnio-centesis (%)	TA-CVS (%)	Difference[†] (95% CI) (%)	Odds ratio[‡] (95% CI)	Odds ratio from Cochrane analysis[11] (95% CI)		
King's College, London[6]	0 (0/731)	0.7 (4/570)	–0.7 (–1.8–0.2)	0 (0–1.2)	0.14 (0–5.4)	Cochrane included only those randomised – these figures not in paper and therefore unavailable for this analysis	Includes 493 women who chose early amniocentesis as well as 239 randomised to early amniocentesis. Includes 320 women who chose CVS as well as 250 randomised to CVS

[*] *Before 13 weeks.*
[†] *Rate of sample failure in early amniocentesis group minus rate in TA-CVS group.*
[‡] *Ratio of odds of sample failure between early amniocentesis and TA-CVS group.*

TABLE 51 *Failure to obtain a sample after TA-CVS compared with TC-CVS*

Trial analysis	Failure to obtain a sample					Main differences between this analysis and Cochrane analysis	Comments on this analysis
	TC-CVS (%)	TA-CVS (%)	Difference[*] (95% CI) (%)	Odds ratio[†] (95% CI)	Odds ratio from Cochrane analysis[11] (99% CI)		
Bologna[7]	23 (14/60)	13 (8/60)	10 (–3.7–23.7)	2.0 (0.8–5.1)	1.0 (0.0–38.8)	Cochrane did not limit number of attempts to obtain sample	Number of attempts to obtain sample limited to two
Milan[8]	0.2 (1/551)	0.2 (1/575)	0 (–0.8–0.8)	1.0 (0.1–15.9)	1.0 (0.0–37.9)	No difference	Authors cite 3.4% of TC-CVS and 2.8% of TA-CVS needed further diagnostic test – not accounted for by summing rates of sampling failure and uninformative results; reason for discrepancy unknown
Philadelphia[9]	2.5 (47/1879)	1.4 (26/1860)	1.1 (0.2–2.0)	1.8 (1.1–2.9)	1.2 (0.8–1.7)	Analysed according to procedure and those who crossed over to other procedure counted as failure to obtain sample regardless of outcome	Only those where assigned procedure carried out are included (figures not available on sample failure for those who crossed over to other procedure). No limit to number of attempts because data not available
Danish (Smidt-Jensen *et al*)[5]	3.1 (44/1419)	1.2 (18/1443)	1.9 (0.8–2.9)	2.5 (1.5–4.4)	2.2 (1.24–4.0)	Cochrane also included samples as failed if too small or failed to grow	
Combined			**1.0 (–0.2–2.1)**	**2.1 (1.5–2.9)**	**1.2 (1.0–1.9)**		

[*] *Rate of sample failure in TC-CVS group minus rate in TA-CVS group.*
[†] *Ratio of odds of sample failure between TC-CVS and TA-CVS groups.*

TABLE 52 *Uninformative result[*] from second trimester amniocentesis compared with no invasive procedure and with CVS*

Trial	Uninformative results							Comments on this analysis
	Amnio-centesis (%)	TC-CVS (%)	TA-CVS (%)	No invasive procedure	Difference[†] (95% CI) (%)	Odds ratio[‡] (95% CI)	Odds ratio from Cochrane analysis[11] (99% CI)	
TC-CVS vs. amniocentesis								
European[2]	0.7 (10/1467)	1.4 (22/1528)	–	–	0.8 (0–1.5)	2.1 (1.0–4.5)	Not reported	Uninformative counted as those who had a second procedure due to laboratory failure, mosaicism, translocations, or maternal cell contamination
Canadian[3]	0.1 (1/968)	2.3 (24/1027)	–	–	2.2 (1.3–3.2)	23.1 (3.1–171.4)	Not reported	Uninformative counted as those reported as 'failure to obtain a laboratory diagnosis when an adequate sample was obtained'
Danish (Smidt-Jensen *et al*)[4]	0.5 (5/961)	1.3 (12/900)	–	–	0.8 (–0.1–1.7)	2.6 (0.9–7.4)	Not reported	Figures obtained from 1991 paper[4] – total number of procedures was slightly lower than 1992 paper;[5] reason unknown. Uninformative counted as those reported as 'inconclusive karyotype'
Combined					**1.2 (0.8–1.7)**	**3.5 (1.3–9.9)**	**Not reported**	
TA-CVS vs. amniocentesis								
Danish (Smidt-Jensen *et al*)[4]	0.5 (5/961)	–	1.4 (14/1018)	–	0.9 (0.0–1.7)	2.7 (1.0–7.4)	Not reported	Figures obtained from 1991 paper[4] – total number of procedures was slightly lower than 1992 paper;[5] reason unknown. Uninformative counted as those reported as 'inconclusive karyotype'

[*] *Due to culture failure, placental mosaicism or maternal cell contamination.*

[†] *Rate of uninformative results in amniocentesis group minus rate in group with no invasive procedure (Tabor* et al[10] *only) or rate of uninformative results in relevant CVS group minus rate in amniocentesis group (other than Tabor).*

[‡] *Ratio of odds of uninformative results between amniocentesis group and group with no invasive procedure (Tabor* et al[10] *only) or between relevant CVS group and amniocentesis group (other than Tabor).*

TABLE 53 *Uninformative result[*] from TA-CVS compared with early amniocentesis[†]*

Trial	Uninformative results					Main difference between this analysis and Cochrane analysis	Comments on this analysis
	Early amnio-centesis (%)	TA-CVS (%)	Differ-ence[‡] (95% CI) (%)	Odds ratio[#] (95% CI)	Odds ratio from Cochrane analysis[11] (95% CI)		
King's College, London[6]	2.5 (18/731)	1.8 (10/566)	0.7 (–0.9–2.3)	1.4 (0.6–3.1)	3.5 (0.6–20.5)	Cochrane included only those randomised – these figures were not in paper and therefore unavailable for this analysis. Cochrane excludes mosaic results from uninformative results	Includes 493 women who chose early amniocentesis as well as 238 randomised to early amniocentesis. Also 320 who chose CVS as well as 250 randomised to CVS. Uninformative results counted as culture failure or mosaic result

[*] *Due to culture failure, placental mosaicism or maternal cell contamination.*
[†] *Before 13 weeks.*
[‡] *Rate of uninformative results in amniocentesis group minus rate in TA-CVS group.*
[#] *Ratio of odds of uninformative results between amniocentesis group and TA-CVS groups.*

TABLE 54 *Uninformative result[*] from TA-CVS compared with TC-CVS*

Trial	Uninformative results					Comments on this analysis
	TC-CVS (%)	TA-CVS (%)	Differ-ence[†] (95% CI) (%)	Odds ratio[‡] (95% CI) (%)	Odds ratio from Cochrane analysis[11]	
Bologna[7]	0 (0/60)	0 (0/60)	Incalculable	Incalculable	Not reported	All cultures successful
Milan[8]	1.5 (9/581)	0.7 (4/575)	0.9 (–0.4–2.1)	2.2 (0.7–7.3)	Not reported	Authors cite 3.4% of TC-CVS and 2.8% of TA-CVS needed further diagnosis – not accounted for by summing rates of sampling failure and uninformative results – reason for discrepancy unknown. Only culture failures reported
Philadelphia[9]	1.0 (19/1832)	1.0 (19/1834)	0 (–0.7–0.7)	1.0 (0.5–1.9)	Not reported	Reasons for diagnostic failure not given
Danish (Smidt-Jensen *et al*)[5]	1.0 (14/1419)	0.6 (9/1443)	0.4 (–0.3–1.0)	1.6 (0.7–3.7)	Not reported	Uninformative results counted as those where a sample was obtained but no diagnosis was achieved
Combined			**0.3 (–0.2–0.7)**	**1.3 (0.8–2.1)**	**Not reported**	**Combined differences and odds ratios based on Milan,[8] Philadelphia[9] and Danish[5] trials**

[*] *Due to culture failure, placental mosaicism, or maternal cell contamination.*
[†] *Rate of uninformative results in TC-CVS group minus rate in TA-CVS group.*
[‡] *Ratio of odds of uninformative results between TC-CVS and TA-CVS groups.*

Chapter 9

Safety and cost-effectiveness of serum screening

It has been shown that multiple marker screening for Down's syndrome is safer and more financially cost-effective than screening based on maternal age alone.[1,2] The decision on which markers to include in a multiple marker serum screening programme should be based on efficacy and cost.

In this chapter we estimate the safety and financial cost-effectiveness of screening using different combinations of serum markers, and also summarise the existing literature on estimated costs associated with antenatal serum screening for Down's syndrome. We estimate the number of induced unaffected foetal losses (attributable to amniocentesis or CVS) for each Down's syndrome birth **avoided** rather than for each Down's syndrome pregnancy **diagnosed**, because this is a more realistic appraisal of the medical costs of screening. The decision to be screened, to have an antenatal diagnosis or to have a termination of pregnancy is a personal choice and the screening service is designed to offer couples this choice. The decisions and actions that follow need to be studied and the service can then be costed realistically. If few women accept screening, the costs and the effect on the birth prevalence of Down's syndrome will be modest, so cost-effectiveness would be little affected. If many women accepted screening, but few decided to have an amniocentesis, cost-effectiveness would be adversely affected.

The lifetime costs of care of a person with Down's syndrome have been estimated by Gill and colleagues.[3] We have not produced a formal comparison of the cost of screening and the costs of lifetime care, because the reason for screening is not to save the costs of care. The purpose is to give couples the opportunity to avoid having a child with a severe abnormality, not to make financial savings for the health services.

Estimates of the parameters used in the cost-effectiveness analysis were as follows.

Screening performance

Estimates of screening performance for different combinations of serum markers were obtained from published sources for the second trimester[4] and the first trimester[5] of pregnancy (see chapters 3 and 7). The false-positive rate was maintained at 5% for all combinations of markers.

Antenatal diagnosis

- The rate of uptake of amniocentesis or CVS after a screen positive result was taken as 80% in unaffected pregnancies and 90% in affected pregnancies (the summary estimates from the demonstration projects in *Tables 21* and *22*, in chapter 5).
- The cost of an amniocentesis was estimated to be £150. This was derived from £120 for the cost of amniocentesis associated with karyotyping (from the North-East Thames Cytogenetics Service), £20 for the costs of the obstetrician, midwife and ultrasonographer (30 minutes for counselling and 15 minutes for the diagnostic procedure), and £10 for consumables and depreciation of equipment. Because the total cost of an amniocentesis is likely to vary between centres, we also used a higher cost of £250 in the cost-effectiveness analysis.
- The cost of CVS was estimated to be £250. This was derived from £210 for the cost of CVS associated with karyotyping (North-East Thames Cytogenetics Service), £30 for the costs of the obstetrician, midwife and ultrasonographer (30 minutes for counselling and 30 minutes for the diagnostic procedure), and £10 for consumables and depreciation. Because the total cost of a CVS is likely to vary between centres we also used a higher cost of £350 in the cost-effectiveness analysis.

Procedure-related unaffected foetal losses

- The foetal loss rate attributable to amniocentesis was taken as 0.9%, with an upper and lower limit of 1.2% and 0.6%, respectively – the estimate from Tabor and colleagues, 1986,[6] and the 25–75th percentile range (see chapter 8). It was assumed that the unaffected foetal loss rate due to transabdominal CVS was similar to that from amniocentesis.
- The cost of removing the retained products of conception after miscarriage due to the invasive diagnostic procedure was taken as £475.[1]

Termination of pregnancy after diagnosis of a foetus with Down's syndrome

- The uptake of termination was taken as 90% (from *Tables 21* and *22*).
- The cost of a termination of pregnancy was taken as £475.[1]

Natural birth prevalence of Down's syndrome and selective foetal loss of affected foetuses

- The birth prevalence of Down's syndrome in the absence of screening was taken as 1.3 per 1000 (derived from OPCS statistics 1984–88[7] and the age-specific risk[8]).
- The spontaneous loss of affected foetuses was taken to be 23% between the second trimester and term,[9] and 48% between first trimester and term.[10]

Cost of providing a serum screening service

Table 55 gives a breakdown of the costs of providing a serum screening test, excluding reagent costs. The interpretation and service costs amount to £3.50 per test; this includes:

(a) staffing costs to process results, administer and monitor the screening service, and train health professionals
(b) provision of information leaflets and interpretive software
(c) office expenses such as stationery, telephone calls, and depreciation of equipment.

The non-reagent laboratory costs amount to about £2.90 per test and are based on those from the screening service performed at the Wolfson Institute of Preventive Medicine, as are reagents costs which are influenced by batch sizes, frequency of assays, and the volume of reagents purchased.

TABLE 55 *Annual costs for providing serum screening service for 10,000 women per year, excluding reagents for double or triple serum tests*

	Cost (£)
Interpretation and service costs	
One full-time data entry clerk	18,750
One third-time screening coordinator	8300
Interpretive software, stationery, printing, fax, telephone, depreciation of equipment	8000
Total per 10,000 women screened	35,050
Cost per woman screened	3.50
Non-reagent laboratory costs	
One full-time technician	18,750
Consumables, equipment, etc.	10,000
Total per 10,000 women screened	28,750
Cost per woman screened	≈ 2.90

Table 56 gives the total cost per test, including reagent costs, according to specified combinations of serum markers and whether assayed in singleton or duplicate. The reagent costs are based on quotes from reagent manufacturers, apart from free α-hCG, which is an in-house assay.

Three areas of costs have not been included.

(a) The cost of collection of the blood sample and transfer to the laboratory was considered to be absorbed into a routine phlebotomy service.
(b) The cost of an ultrasound dating scan was considered to be part of existing routine obstetric care and so was not an extra cost associated with screening. We have specified estimates of efficacy, safety, and the financial costs associated with screening according to method of estimation of gestational age, which applies to a particular maternity unit. The benefit of using an ultrasound scan examination to estimate gestational age is greatest for those combinations of markers which include uE_3, because the concentration of uE_3 changes most with gestational age.
(c) The cost of the midwives' time in giving information before the test and reporting screen negative results was considered to be absorbed into their routine work. Information before the test about serum screening is usually given at the booking visit, at about 12 weeks of pregnancy, with other information on care during pregnancy, and so it should not normally increase the total time devoted to this consultation. It is important that the implications of the screening tests are clear at the outset, and written information should be provided to supplement oral information. The cost of 30 minutes' counselling after a screen positive result was included and estimated to be £10, and this was included in the costs of antenatal diagnosis and added separately if antenatal diagnosis was declined.

Safety or 'medical cost-effectiveness' was expressed in terms of the number of unaffected foetuses lost owing to amniocentesis or CVS for each Down's syndrome birth avoided – the smaller the ratio, the more favourable the medical cost-effectiveness. The estimates were based on screening women in whom the birth prevalence is 1.3 per 1000 in the absence of screening. *Figure 23* illustrates the calculations using maternal age with AFP and free β-hCG,

TABLE 56 *Financial cost-effectiveness of second trimester serum screening: cost per serum screening test, including reagent costs for assaying in singleton and duplicate, according to specified combinations of serum markers*

Screening test[†]	Interpretation and service costs (£)	Non-reagent laboratory costs (£)	Reagent costs for singleton assays[*] (£)							Total cost (singleton assay)	Total cost (duplicate assay)
			AFP	Free β-hCG	hCG	uE_3	Free α-hCG	Inhibin A	PAPP-A		
Second trimester											
AFP, free β-hCG	3.50	2.90	1.10	1.40	–	–	–	–	–	8.90	11.40
AFP, total hCG	3.50	2.90	1.10	–	1.10	–	–	–	–	8.60	10.80
AFP, uE_3, free β-hCG	3.50	2.90	1.10	1.40	–	1.00	–	–	–	9.90	13.40
AFP, uE_3, total hCG	3.50	2.90	1.10	–	1.10	1.00	–	–	–	9.60	12.20
AFP, uE_3, free β-hCG, free α-hCG	3.50	3.40[‡]	1.10	1.40	–	1.00	0.10	–	–	10.50	14.10
AFP, uE_3, free β-hCG, inhibin A	3.50	3.40[‡]	1.10	1.40	–	1.00	–	1.50	–	11.90	16.90
AFP, uE_3, total hCG, inhibin A	3.50	3.40[‡]	1.10	–	1.10	1.00	–	1.50	–	11.60	16.30
First trimester											
PAPP-A, free β-hCG	3.50	2.90	–	1.40	–	–	–	–	1.60	9.40	12.40

[*] *If assaying in duplicate the reagent costs are doubled.*
[†] *All with maternal age.*
[‡] *Extra £0.50 for extra labour costs associated with free α-hCG and inhibin A assays.*

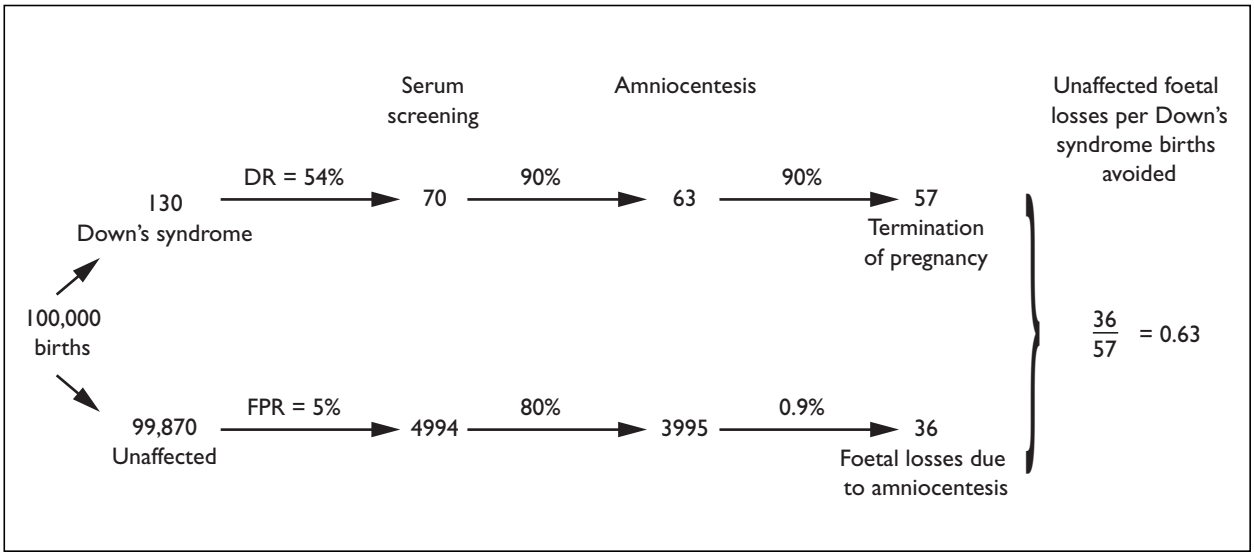

FIGURE 23 *Calculation of the number of procedure-related unaffected foetal losses per Down's syndrome birth avoided in 100,000 births using AFP and free β-hCG with gestation estimated by dates and a 0.9% procedure-related foetal loss rate in unaffected pregnancies*

gestational age estimated by dates (54% detection rate for a 5% false-positive rate) and a procedure-related foetal loss rate of 0.9%. (A formal method of calculation is given at the end (page 83) of this chapter.)

Table 57 shows the detection rate for a 5% false-positive rate and the unaffected foetal losses per Down's syndrome pregnancy avoided according to different combinations of serum markers in the second trimester, method of estimating gestational age (dates or scan), and the procedure-related foetal loss rate. The least effective combination is the double test and the most effective combination is AFP, uE_3, total hCG, and inhibin A. If the procedure-related foetal loss rate is taken to be 0.9%, and gestational age is estimated by ultrasound scan, there would be 0.45 unaffected foetal losses per Down's syndrome birth avoided using AFP, uE_3, total hCG, and inhibin A compared with 0.59 (32% more) with AFP and free β-hCG.

TABLE 57 *Safety of second trimester serum screening: unaffected foetal losses per Down's syndrome birth avoided according to specified combinations of serum markers and whether gestational age was estimated by dates or by ultrasound scan*

Method of screening: maternal age with†	DR (%) for a 5% FPR*		Unaffected foetal losses per Down's syndrome birth avoided					
			0.6% procedure-related foetal loss rate		0.9% procedure-related foetal loss rate		1.2% procedure-related foetal loss rate	
	Dates	Scan	Dates	Scan	Dates	Scan	Dates	Scan
AFP, free β-hCG	54	58	0.42	0.39	0.63	0.59	0.84	0.78
AFP, total hCG	54	59	0.42	0.39	0.63	0.58	0.84	0.77
AFP, uE_3, free β-hCG	60	68	0.38	0.33	0.57	0.50	0.76	0.67
AFP, uE_3, total hCG	59	69	0.39	0.33	0.58	0.49	0.77	0.66
AFP, uE_3, free β-hCG, free α-hCG	65	73	0.35	0.31	0.52	0.47	0.70	0.62
AFP, uE_3, free β-hCG, inhibin A	67	75	0.34	0.30	0.51	0.45	0.68	0.61
AFP, uE_3, total hCG, inhibin A	67	76	0.34	0.30	0.51	0.45	0.68	0.60

* *Taken from Wald* et al, *1996*[4] *and chapter 3.*
† *Serum marker levels corrected for maternal weight.*

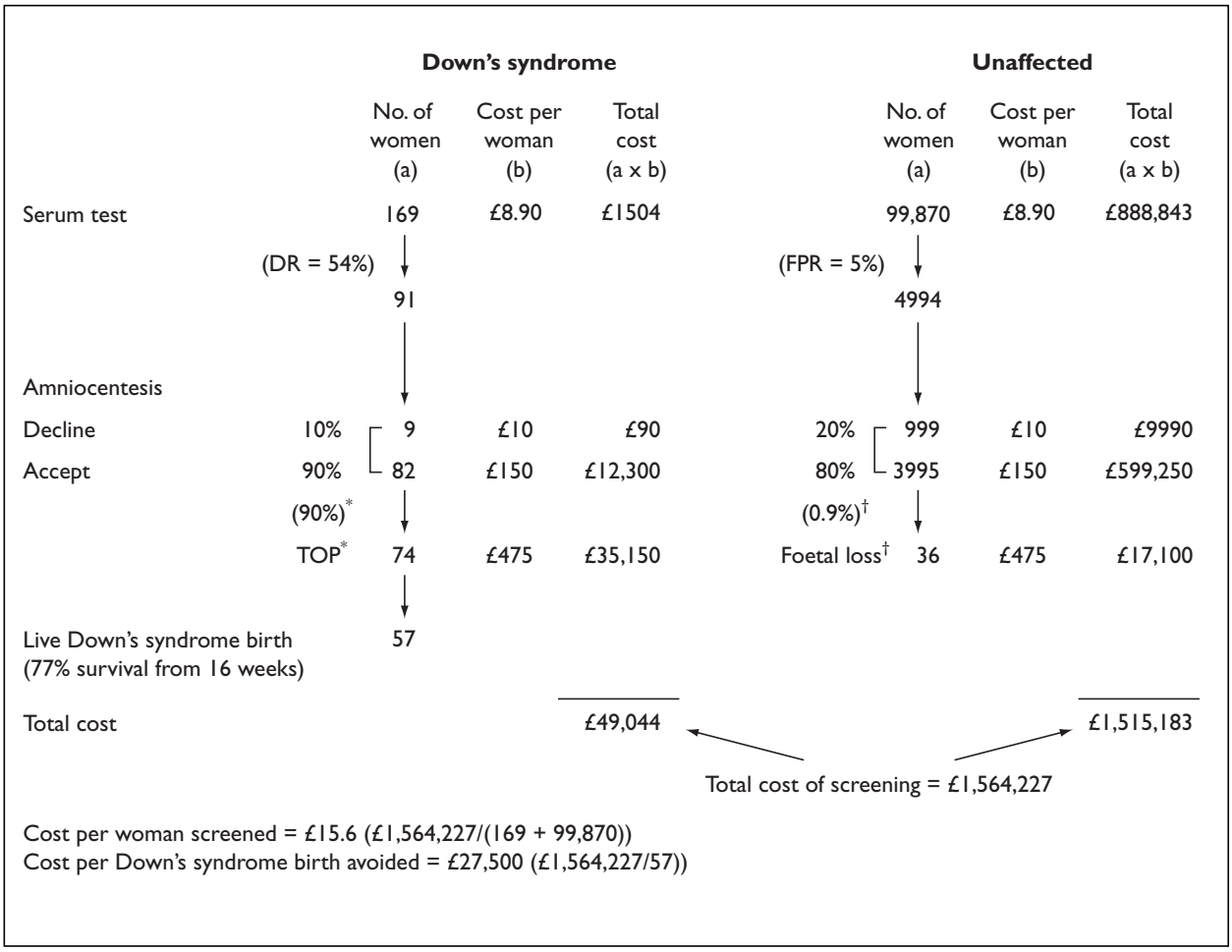

FIGURE 24 *Calculation of the cost per woman screened at 15–22 weeks and cost per Down's syndrome birth avoided in 100,000 term pregnancies using AFP and free β-hCG with gestation estimated using singleton assays and £150 for an amniocentesis. The calculation assumes there are 130 Down's syndrome births in 100,000 (1.3 per 1000) and a corresponding mid-trimester prevalence of 169 affected pregnancies (130/0.77), where 0.77 is the survival rate from 16 weeks to term. (Cost of removal of foetal products is £475.)*
* *TOP = termination of affected pregnancy (90%).* † *Foetal loss = the procedure-related foetal loss in unaffected pregnancies (0.9%)*

The financial cost-effectiveness was expressed in terms of the cost per Down's syndrome birth avoided – again, the smaller the ratio, the more favourable the financial cost-effectiveness. *Figure 24* illustrates the calculation, including the cost per woman screened. (See page 83 for formal calculation.)

Table 59 shows the financial costs per woman screened, together with the cost per Down's syndrome birth avoided, for different combinations of serum markers in the second trimester, according to the method of estimating gestational age (dates or scan) and according to the cost of amniocentesis. Although the total costs increase as extra serum markers are added, the cost per Down's syndrome birth avoided tends to decrease because of the increase in the detection rate. If the amniocentesis cost is taken to be £150, gestational age is estimated by ultrasound scan and samples are assayed in singleton, it would cost £23,100 to avoid one Down's syndrome birth using AFP, uE_3, total hCG and inhibin A, compared with £25,600 with AFP and free β-hCG, making the former the most cost-effective as well as the most effective method of screening.

Figure 25 shows the average cost per Down's syndrome birth avoided and the false-positive rate in relation to the detection rate for different combinations of serum markers used. To achieve up to a 35% detection rate, maternal age screening is the most cost-effective screening method (see Wald & Watt, 1996, *Figure 1*[11]). At detection rates of about 65% or more, the quadruple test is the most cost-effective. In making these comparisons of cost-effectiveness between different test combinations, it is necessary to hold the detection rate constant, otherwise costs are not being compared for the same outcome. The relative cost-effectiveness of different methods of screening differs according to the detection rate. This argument has been set out before.[11]

We believe that the average cost per Down's syndrome birth avoided (or Down's syndrome pregnancy diagnosed) is the appropriate cost estimate to use when comparing different methods of screening. Marginal cost estimates are the relevant ones to use in determining whether, from a financial point of view, it is worth lowering the risk cut-off level so that using a given method of screening the detection rate can be increased. For example, in screening 100,000 women using the triple test, decreasing the risk cut-off level from 1:250 to 1:350 would cost an extra £230,000

TABLE 58 *Financial cost-effectiveness of serum screening in the second trimester, according to specified combinations of serum markers (corrected for maternal weight) and gestational age estimated by dates and by ultrasound scan, with a procedure-related foetal loss rate of 0.9%*

Method of screening: maternal age with	Cost of serum screening test (£)	Amniocentesis cost £150				Amniocentesis cost £250			
		Cost per woman screened (£)		Cost per Down's syndrome birth avoided (£000)		Cost per woman screened (£)		Cost per Down's syndrome birth avoided (£000)	
		Dates	Scan	Dates	Scan	Dates	Scan	Dates	Scan
Assays performed in singleton									
AFP, free β-hCG	8.90	15.6	15.7	27.5	25.6	19.7	19.8	34.6	32.3
AFP, total hCG	8.60	15.3	15.4	27.0	24.7	19.4	19.5	34.1	31.3
AFP, uE_3, free β-hCG	9.90	16.7	16.8	26.4	23.4	20.8	20.9	32.9	29.1
AFP, uE_3, total hCG	9.60	16.4	16.5	26.3	22.7	20.5	20.6	32.9	28.3
AFP, uE_3, free β-hCG, free α-hCG	10.50	17.3	17.4	25.3	22.6	21.4	21.5	31.3	28.0
AFP, uE_3, free β-hCG, inhibin A	11.90	18.7	18.8	26.6	23.8	22.8	22.9	32.4	29.0
AFP, uE_3, total hCG, inhibin A	11.60	18.4	18.5	26.1	23.1	22.5	22.6	31.9	28.3
Assays performed in duplicate									
AFP, free β-hCG	11.40	18.1	18.2	31.9	29.7	22.2	22.3	39.0	36.4
AFP, total hCG	10.80	17.5	17.6	30.8	28.3	21.6	21.7	38.0	34.8
AFP, uE_3, free β-hCG	13.40	20.2	20.3	31.9	28.3	24.3	24.4	38.4	34.0
AFP, uE_3, total hCG	12.20	19.0	19.1	30.5	26.2	23.1	23.2	37.1	31.9
AFP, uE_3, free β-hCG, free α-hCG	14.10	20.9	21.0	30.6	27.3	25.0	25.1	36.5	32.6
AFP, uE_3, free β-hCG, inhibin A	16.90	23.7	23.8	33.6	30.1	27.8	27.9	39.4	35.3
AFP, uE_3, total hCG, inhibin A	16.30	23.1	23.2	32.7	29.0	27.2	27.3	38.6	34.1

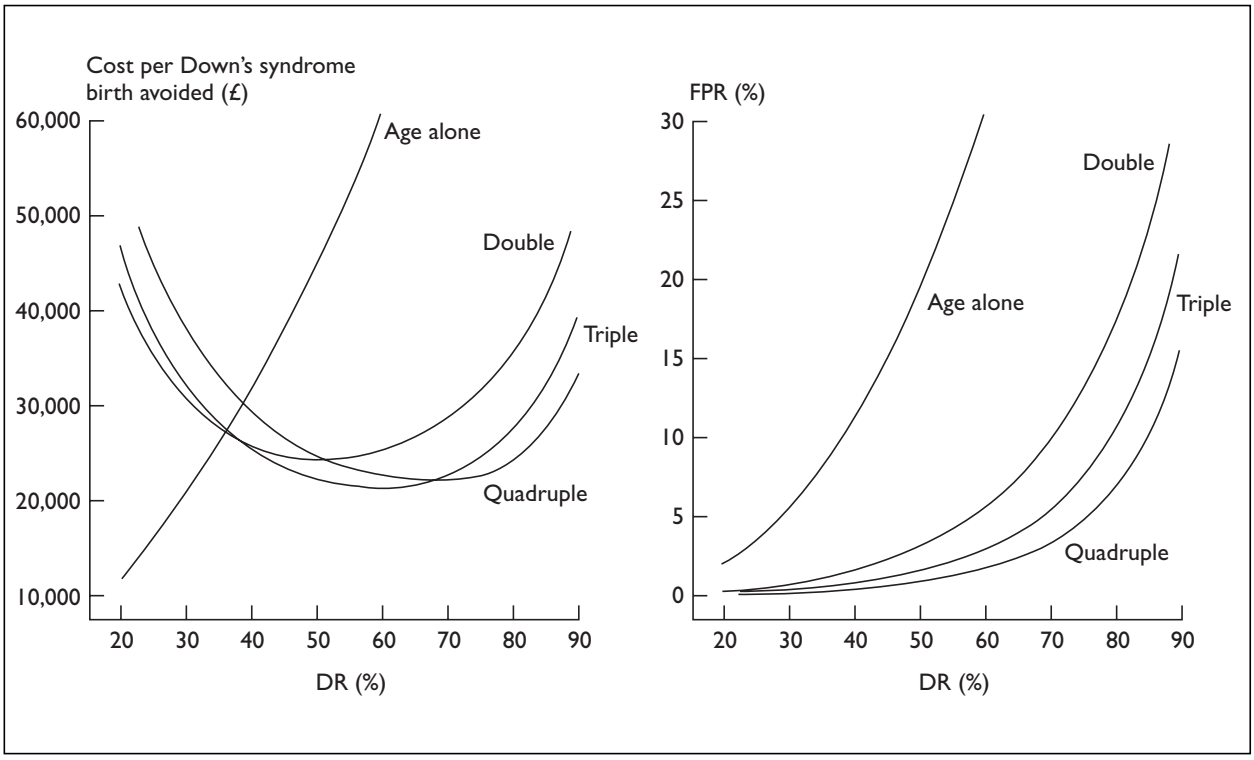

FIGURE 25 *Comparison of the cost per Down's syndrome birth avoided with screening performance using maternal age alone, the double test (AFP and total hCG), triple test (AFP, uE_3 and total hCG) and the quadruple test (AFP, uE_3, total hCG and inhibin A). Costs assume scan gestation, singleton assays, £150 for an amniocentesis, and a procedure-related foetal loss rate of 0.9% in unaffected pregnancies*

because of the extra amniocenteses needed, and lead to an extra five Down's syndrome births avoided at a marginal cost per extra case avoided of about £46,000.

If this were felt to be too great a cost for the extra benefit, the cut-off level need not be altered. There are thus two distinct questions in cost-effectiveness calculations for screening. Firstly, which method of screening is the least expensive method of achieving a given detection rate. This should be determined from average costs per affected birth avoided or case diagnosed.

Secondly, what is the extra cost of increasing the detection rate by a specified amount when a particular method of screening is being used. This should be determined from marginal costs.

Figure 26 compares both the safety and financial cost-effectiveness of screening using different combinations of serum markers (all with maternal age).

The estimates of cost-effectiveness and safety are given for each Down's syndrome birth avoided. The estimates will be more favourable if the calculations are given for each Down's syndrome pregnancy diagnosed *(Table 59)*.

Table 60 shows the safety and financial cost-effectiveness of serum screening in the first trimester using maternal age with free β-hCG and PAPP-A, according to the cost of CVS and the foetal loss rate due to CVS. If the latter is taken to be 0.9%, there would be 0.55 unaffected foetal losses per Down's syndrome birth avoided – similar to that using the double or triple test in the second trimester, using gestational age estimated by scan (see *Table 57)*. If the cost of CVS is taken to be £250, and assays are performed in singleton, the cost per Down's syndrome birth avoided would be £31,600 – more expensive than any form of second trimester serum screening if the amniocentesis cost is taken to be £150 (see *Table 58*).

We have carried out cost-effectiveness analyses with assays that have been performed either in singleton or in duplicate, the latter always being more expensive and less cost-effective. The use of duplicate assays reduces the standard deviation of the serum marker levels, thereby improving screening performance. Using data from Bart's and the triple test (maternal age with AFP, uE_3, and free β-hCG), we estimated that at a 5% false-positive rate, the detection rate would increase by only about 1% (69% compared with 68%) when using duplicate assays instead of singleton assays. The effect on cost is too small to be worthwhile in relation to the total cost.

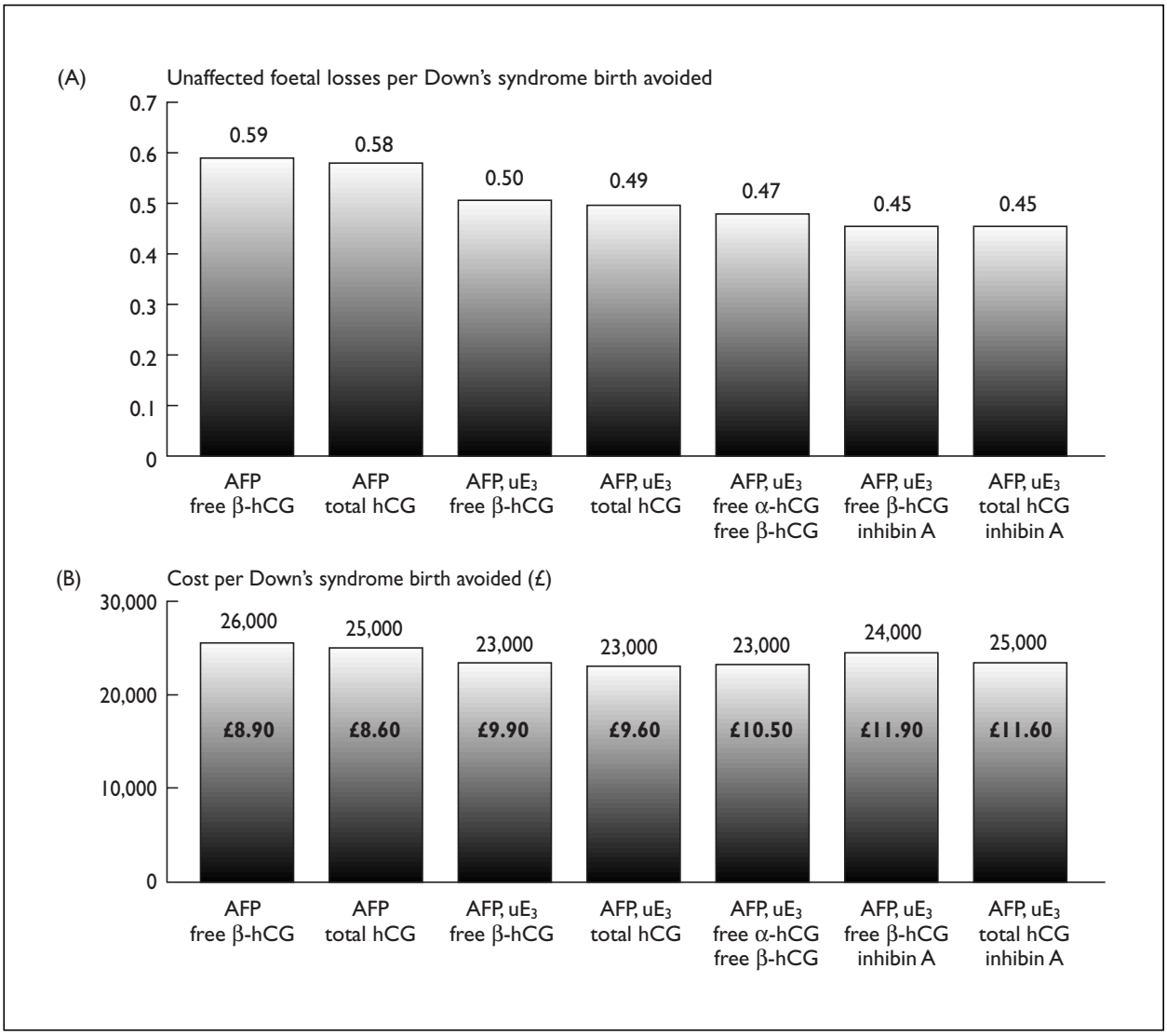

FIGURE 26 *Comparison of the safety (A) and financial cost-effectiveness (B) of different combinations of serum markers using singleton assays, scan gestation, £150 for an amniocentesis, and a procedure-related foetal loss rate of 0.9% in unaffected pregnancies. The cost of the serum test per woman is indicated in the bars of the lower bar chart*

TABLE 59 *Financial cost-effectiveness and safety of second trimester screening in relation to the number of Down's syndrome pregnancies detected*

Method of screening: maternal age with	Cost per Down's syndrome pregnancy detected (£000)		Unaffected foetal losses per Down's syndrome pregnancy detected	
	Dates	Scan	Dates	Scan
AFP, free β-hCG	19.0	17.8	0.44	0.41
AFP, total hCG	18.7	17.1	0.44	0.40
AFP, uE_3, free β-hCG	18.3	16.2	0.39	0.35
AFP, uE_3, total hCG	18.3	15.7	0.40	0.34
AFP, uE_3, free β-hCG, free α-hCG	17.5	15.7	0.36	0.32
AFP, uE_3, free β-hCG, inhibin A	18.4	16.5	0.35	0.32
AFP, uE_3, total hCG, inhibin A	18.1	16.0	0.35	0.31

Assays performed in singleton; amniocentesis cost of £150; procedure-related foetal loss rate of 0.9%.

TABLE 60 *Financial cost-effectiveness of serum screening in the first trimester and unaffected foetal losses per Down's syndrome birth avoided using maternal age with free β-hCG and PAPP-A at a cost per test of £9.40 (singleton assay) and £12.40 (duplicate assay) (DR 62% for a 5% FPR)*[5]

Cost of CVS (£)	Cost per woman screened* (£)		Cost per Down's syndrome birth avoided* (£000)		Unaffected foetal losses per Down's syndrome birth avoided		
	Singleton assays	Duplicate assays	Singleton assays	Duplicate assays	0.6% procedure-related foetal loss rate	0.9% procedure-related foetal loss rate	1.2% procedure-related foetal loss rate
250	20.6	23.6	31.6	36.2	0.37	0.55	0.73
350	24.7	27.7	37.9	42.5	0.37	0.55	0.73

* *Based on an unaffected foetal loss rate of 0.9%.*
Calculations based on 100,000 births in which there are 130 with Down's syndrome, corresponding to 250 affected pregnancies at 10–14 weeks (130/0.52 where 0.52 is the survival rate from 10–14 weeks to term).

TABLE 61 *Marginal financial cost-effectiveness of serum screening in the second trimester, assuming the existence of an AFP screening programme for NTDs, with a procedure-related loss rate of 0.9% in unaffected pregnancies, amniocentesis cost of £150 and samples assayed in singleton*

Method of screening: maternal age with	Cost of serum screening test* (£)	Cost per woman screened (£)		Cost per Down's syndrome birth avoided (£000)	
		Gestation estimated by		Gestation estimated by	
		Dates	Scan	Dates	Scan
AFP, free β-hCG	5.90	12.6	12.7	22.2	20.7
AFP, total hCG	5.60	12.3	12.4	21.7	19.9
AFP, uE_3, free β-hCG	6.90	13.7	13.8	21.6	19.2
AFP, uE_3, total hCG	6.60	13.4	13.5	21.5	18.5
AFP, uE_3, free β-hCG, free α-hCG	7.50	14.3	14.4	20.9	18.7
AFP, uE_3, free β-hCG, inhibin A	8.90	15.7	15.8	22.3	20.0
AFP, uE_3, total hCG, inhibin A	8.60	15.4	15.5	21.9	19.4

* *Calculated as the total cost per test in* Table 57 *minus £3, the estimated cost associated with NTD screening.*

An additional benefit of offering Down's syndrome serum screening is the provision of screening for NTDs using AFP, although its value over routine second trimester ultrasound is becoming less certain. In many health districts an AFP screening service for NTDs has existed for many years. The financial cost-effectiveness of Down's syndrome serum screening is improved if the existence of an AFP screening service for NTDs is taken into account. Some of the costs associated with providing a Down's syndrome screening service are already incurred in an NTD screening programme, and are estimated as half of a full-time laboratory technician, £10,000 laboratory expenses for screening 10,000 women per year, and AFP reagent costs (£1.10 per woman). This amounts to about £3 per screening test if samples are assayed in singleton (which can be subtracted from the total cost per test in *Table 56*). *Table 61* shows the marginal financial cost-effectiveness of Down's syndrome serum screening, assuming an existing AFP service, giving the costs per woman screened, together with the costs per Down's syndrome birth avoided. If the quadruple test (AFP, uE_3, total hCG, and inhibin A) is used, gestational age is estimated by ultrasound scan and the cost of an amniocentesis is £150, the cost per Down's syndrome birth avoided is reduced by 16% (from £23,100 to £19,400 *(Tables 58* and *61))*, if the existence of an AFP screening programme is taken into account.

Table 62 summarises the published literature on estimated costs associated with antenatal screening for Down's syndrome in the UK.[12–18] The median cost of the triple test from five studies in which this information was given (using only the most recent cost estimate from Wald *et al*[17]) was £15.41. The median cost of amniocentesis from the eight

TABLE 62 *Published studies of the cost (£) of antenatal screening for Down's syndrome**

	Hagard 1976[12]	Gill, 1987[3]	Sheldon, 1991[2]	Wald, 1992[13]	Shackley, 1993[1]	Piggott, 1994[14]	Wald, 1994[15]	Wessex, 1994[16]	Wald, 1994[17]	Fletcher, 1995[18]
Serum screening										
Consumables					3.86					
Staff					8.90					
Communication					0.28					
Maintenance					0.35					
Computer software					0.12					
Transport					0.04					
Capital					0.81					
Procedure:										
Cytogenetic analysis										
Triple test (total)			11.42		14.36	31.61	15.81		15.41	13.70
Double test (total)							12.12		12.32	
Quadruple test (total)									16.95	
Ultrasound		6.90						19.63		
Diagnostic testing										
Consultant time (including counselling)		13.08								
Disposables		6.99								
Nursing sister time		2.69								
Transport		10.00								
Procedure:										
Laboratory costs		103.52								
Amniocentesis (total)	813.97	136.29	108.47	163.59	263.43	210.74	158.06	223.57	154.05	250.00
Therapeutic/ spontaneous abortion			1084.75	1090.58	474.17			468.95		450.00

* *Costs adjusted to 1995 prices, after taking account of inflation.*
Studies identified by first author only to save space.

studies (using only the most recent cost estimate from Wald *et al*[17]) was about £220, between our two estimates of £150 and £250. The median cost of termination of pregnancy was £475, the estimate used in our calculations.

First trimester serum screening is reasonably effective and safe, but it is less effective and less cost-effective than second trimester screening (see *Table 60*). There is no published literature on the cost-effectiveness of ultrasound screening alone or combined with serum screening in the first trimester, but this is likely to be more expensive than serum screening if an ultrasound examination has not already been done; it may be less expensive if a scan is performed routinely, in which case the cost is that arising from the extra time required for obtaining a nuchal translucency measurement. A disadvantage of first trimester screening is that it precludes screening for NTDs, which is performed after 15 weeks of pregnancy.

Calculations

The formal methods of calculation are as follows.

(1) The unaffected foetal losses per Down's syndrome birth avoided

(1a) Number of Down's syndrome births avoided = (number of affected births screened) × (detection rate) × (uptake of antenatal diagnosis) × (uptake of termination)

(1b) Number of unaffected foetal losses = (number of unaffected births screened) × (false-positive rate) × (uptake of antenatal diagnosis) × (procedure-induced foetal loss rate)

The unaffected foetal losses per Down's syndrome birth avoided are therefore (1b)/(1a).

(2) The cost of screening per woman

(2a) Cost of screening affected pregnancies = (number of affected pregnancies screened × cost of serum test) + (number of true-positives × uptake of antenatal diagnosis × cost of antenatal diagnosis) + (number of true positives × % declining antenatal diagnosis × cost of counselling) + (number of true

positives × uptake of antenatal diagnosis × uptake of termination × cost of termination)

(2b) Cost of screening unaffected pregnancies = (number of unaffected pregnancies screened × cost of serum test) + (number of false-positives × uptake of antenatal diagnosis × cost of antenatal diagnosis) + (number of false-positives × % declining antenatal diagnosis × cost of counselling) + (number of false-positives × uptake of antenatal diagnosis × procedure-induced foetal loss rate × cost of procedure-induced foetal loss)

The cost of screening each woman is therefore (2a + 2b)/(number of affected pregnancies screened + number of unaffected pregnancies screened).

(3) The cost per Down's syndrome birth avoided

Cost per Down's syndrome birth avoided = (cost of screening affected pregnancies + cost of screening unaffected pregnancies)/number of Down's syndrome births avoided (that is, (2a + 2b)/1a).

- The number of true-positives = number of affected pregnancies screened × detection rate.
- The number of false-positives = number of unaffected pregnancies screened × false-positive rate.

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Chapter 10

Psychosocial aspects

Screening necessarily causes anxiety because it identifies individuals with a high risk of a serious medical disorder. It effectively creates patients from the general population of pregnant women by identifying some as being at increased risk. A positive screening result makes the awareness of the risk real and personal at a particularly poignant time because of the strong emotions associated with pregnancy. Screening makes uncertainty explicit, and this itself can be distressing.

It is the duty of the screening service to provide appropriate information and personal support so that the anxiety generated by screening is constructive and temporary – helping women to make decisions that they feel are right for them. Screening should not be confined to simply performing tests and reporting the results. Screening is often seen as a means of reassurance, rather than a means of identifying abnormalities, and is apt to raise expectations that a negative screening result rules out the possibility of an affected pregnancy. This must be guarded against.

Women need appropriate knowledge of Down's syndrome and how it affects the child and adult, both mentally and physically. While there are exceptions, the prognosis is poor. Down's syndrome is the most common cause of severe mental retardation. By the age of 21, the mean IQ is about 42 (ranging from 8 to 67) – a mean mental age of 5 years[1]; 40% can read (with a mean reading age of 8 years). About 40% of affected adults aged 21 can feed, dress, wash, and go to the toilet, all without help, and about 17% can be left in the home unattended. Down's syndrome is also associated with major physical problems. About 50% of babies born with Down's syndrome have at least one serious congenital abnormality,[2] the most common being heart defects (about 45%) and gastrointestinal defects (about 6%). Of those with congenital heart defects, an estimated 40% are expected to die before the age of 5 years.[3] About 75% of those with gastrointestinal defects have atresia of the small intestine, which requires surgery and, possibly, colostomies.[4] People with Down's syndrome are at a higher risk of leukaemia,[3] cerebral palsy,[3] hydrocephalus,[3] hypothyroidism,[5,6] epilepsy,[7] and Alzheimer's disease.[8]

About four out of five children with Down's syndrome survive to the age of 5 years, and of these survivors, about 40% are likely to have major health problems (other than mental retardation).[3] The average life expectancy is about 60 years (compared with 75–80 years in the general population).[9,10]

Women need appropriate knowledge of the screening test, together with the limitations of the test, so that they can decide whether they wish to be screened. They need to consider their possible action if a test result suggests that antenatal diagnosis is indicated and if Down's syndrome is subsequently diagnosed. It is important for them to recognise that a screen positive result does not necessarily mean that their pregnancy is affected and that a screen negative result does not provide reassurance that they are no longer at risk.

Much that is often considered as the 'psychosocial' aspect of screening concerns the self-evident need to provide a well-informed, compassionate service that respects the wishes of individuals. Nevertheless, it is often in this area that screening fails and causes 'casualties' that could, with appropriate care, be avoided. Various studies have examined women's experiences of serum screening and others have examined the activities of the health professionals who help to provide the service. In this chapter we summarise the studies and try to bring together the main conclusions. Most of the studies relate to screening for Down's syndrome using maternal age and AFP alone, but the findings are also applicable to multiple marker screening.

Table 63 shows studies which assessed experiences of women being screened. Anxiety was assessed in most of the studies but in many it was not acknowledged that anxiety is a necessary cost of realising that there is an increased risk of a serious disease or a foetal abnormality. Often the studies have simply confirmed that antenatal serum screening causes anxiety. Some authors have viewed this as an adverse finding. This is only the case if the anxiety is excessive and could have been appropriately avoided. Our review indicates that, in general, the anxiety caused by screening is short-lived and resolves after a favourable amniocentesis result.

TABLE 63 *Serum screening for Down's syndrome: a review of studies on women's experiences*

Study	Screening test	Study participants	Methods	Results	Main conclusions
Abuelo *et al*, 1991[11]	AFP for DS	• 50 offered amniocentesis because of low AFP (all < 35 years) • 50 offered amniocentesis because of age (≥ 35 years)	Anxiety measured using Spielberger's state–trait anxiety inventory. Three stages: (1) before amniocentesis counselling; (2) after amniocentesis counselling; (3) after normal karyotype	No difference in anxiety between two groups after normal karyotype; significant increase in anxiety in group with low AFP over maternal age group at stages 1 and 2	Abnormal screening result in a woman previously thought not to be at increased risk of birth defects was associated with development of significant anxiety
Keenan *et al*, 1991[12]	AFP for DS	• 52 with low AFP results • 25 controls (low risk)	Anxiety measured using Spielberger's state–trait anxiety inventory, once in controls and twice in group with low AFP, before and immediately after amniocentesis counselling	Anxiety lower in controls than group with low AFP both before and after counselling stages. Anxiety lower in group with low AFP after counselling	Counselling reduced anxiety in women with low AFP whether or not amniocentesis performed
Marteau *et al*, 1992[13]	AFP for DS and NTDs	• 372:346 with normal AFP, 10 with raised AFP, 16 with low AFP (637 excluded for failure to complete questionnaires)	Anxiety measured using Spielberger's state–trait anxiety inventory. Seven stages, one before and six after AFP testing. Questionnaire included attitudes towards pregnancy and baby and concerns over baby's health	Those with abnormal result more anxious after result and 3 weeks later; also more concerned about baby's health; women who had amniocentesis less worried about baby's health in third trimester and *post partum*	Abnormal AFP result was associated with high levels of anxiety. No evidence of abnormal result having an effect on anxiety later in pregnancy (that is, at 26 weeks, 36 weeks and after delivery)
Statham, & Green 1993[14]	DS serum screening; policy N/S	20 women with screen positive results who had contacted Support After Termination for Abnormality (NB: This group may not be representative of all those with positive results)	Semi-structured interview and correspondence on understanding of screening test, staff misconceptions, communication of results, coping with diagnostic process, attitudes to screening, and termination for abnormality; four interviews immediately after screening, eight after negative amniocentesis, eight after termination for abnormality	All women made anxious by positive result no matter how informed; some remained anxious even after negative amniocentesis. Medical staff unclear about test implications and how to interpret risk; staff did not always recognise women's concerns while waiting for amniocentesis results	Implementation of serum screening did not always meet the needs of women with positive results; appropriate support for screening test participants should have been adopted at outset
Roelofsen *et al*, 1993[15]	AFP, hCG	105 women out of 200 women approached, < 36 years who had given birth in the past 4 months. A second group excluded from our analysis had no direct serum screening experience	Questionnaire on experiences, knowledge, attitudes, intentions, and motives regarding serum screening and its implications, information on screening and possible subsequent tests, procedures and risks included in questionnaires	80% serum screened (two found to be at increased risk), of whom > 70% would have accepted amniocentesis if at increased risk; 81% would have screening again in future; 32% had test for reassurance, 26% because it was the "obvious thing to do", 65% not aware of possible drawbacks, 60% satisfied with information	Serum screening was often seen as a means of reassurance and women often not aware of possible drawbacks
Marteau *et al*, 1993[16]	AFP for DS and NTDs	Women presenting at < 16 weeks of pregnancy randomised to five groups; three received intervention (one a detailed booklet on the AFP test, one offered early antenatal class in anxiety management, one offered both), two control groups, one for intervention group, one for questionnaire completion	The three intervention groups and one of the control groups completed questionnaires at different times during pregnancy – the first before any testing and the last in the third trimester. Multiple choice questionnaire (MCQ) on knowledge of AFP test, influence of booklet or antenatal class, satisfaction with information. Anxiety measured using Spielberger's state–trait anxiety inventory	85 women had FPRs (high and low AFP). No evidence that abnormal AFP increased anxiety (in contrast with previous studies). Detailed written information led to women having more knowledge and more satisfaction with information. Neither intervention alone or in combination had an effect on anxiety after an abnormal AFP result	Providing women with written detailed information would have been useful

N/S = not specified; DS = Down's syndrome; MCQ = multiple choice questionnaire.

continued

TABLE 63 contd *Serum screening for Down's syndrome: a review of studies on women's experiences*

Study	Screening test	Study participants	Methods	Results	Main conclusions
Kidd *et al,* 1993[17]	AFP for DS and NTDs	Women < 38 years presenting for antenatal care at < 16 weeks of pregnancy. 309 had been screened, 30 had not	Questionnaires completed at four stages covering attitudes to baby's health, knowledge of screening test. Anxiety measured using Spielberger's state–trait anxiety inventory	21/309 women screened thought they had not been; 7/30 not screened thought they had been; no difference between the two groups for anxiety, certainty, or worry about the baby's health	Screen negative result did not provide reassurance
Santalahti *et al,* 1996[18]	DS serum screening; policy N/S	45 case women with positive serum screening results (two had an affected pregnancy and one miscarried after amniocentesis), 46 control women either with negative screening results or not screened, matched for age, parity, education, and previous miscarriages	Semi-structured interview on women's experience of pregnancy, particularly regarding serum screening	Most of the 33 case women who had unaffected pregnancies and had amniocentesis or CVS were distressed by the screening result and six of these were still worried after receiving final reassuring results. The seven case women who declined antenatal diagnosis were not significantly distressed. Two case women who received negative serum screening results after the first positive result remained worried. Of the 46 control women, 17 felt some worry or fear about abnormality in their baby	Receiving a positive serum screening result had a negative influence on women's pregnancy experiences
Smith *et al,* 1994[19]	DS serum screening; policy N/S	353 women < 8 weeks' gestation who attended one of five UK hospitals offering routine serum screening for DS	Women completed MCQ of nine questions assessing knowledge of screening test after a consultation with a midwife or obstetrician during which DS was discussed	Women were knowledgeable about practical aspects of the test (72% knew it was a blood test and 89% knew it was performed between 16 and 18 weeks of pregnancy); women were less informed about test implications (32% knew that most women with positive results have normal babies and 38% knew that the test screened for DS)	Screening programme did not address the information needs of women adequately, particularly the implications of possible screening results, including the likelihood of a screen positive result
Thornton *et al,* 1995[20]	DS serum screening; policy N/S	Women booking for antenatal care at < 15 weeks, randomised into one of three groups; control group (n = 587) given routine information given on screening test by midwife or doctor at time of booking and an information sheet on antenatal screening; individual information group (n = 561) offered extra individual consultation by specially trained staff and also given information leaflet; class information group (n = 563) offered extra visit in groups of 4–12 using specially trained staff and also given information leaflet	Questionnaires by post for self-completion at 16–18 weeks, 20 weeks, 30 weeks, and 6 weeks after delivery included Spielberger's state–trait anxiety inventory, hospital anxiety and depression scale, questionnaire on knowledge and understanding, anxiety measure specific to pregnancy and foetal abnormality	Women offered extra information had improved understanding and were more satisfied with information. No difference in satisfaction with decisions about antenatal diagnosis. Offer of individual information reduced anxiety later in pregnancy and had no adverse effects. Offer of extra information reduced uptake of screening when background rate was high, but not when it was already low	Enhanced provision of information was particularly important when a new screening test was introduced, because the risk of compliant behaviour was highest at this stage. Giving antenatal screening information in classes was not popular. Offering healthy people more information did not increase anxiety overall. High uptake of screening tests suggested compliant behaviour and need for more information

N/S = not specified; DS = Down's syndrome; MCQ = multiple choice questionnaire.

The studies have shown that, in general, women are aware that they are being screened and are given a choice of whether to be screened. Most screening centres provide written information, and this has been found to be useful. Two studies showed that women are often falsely reassured by a screen negative result. One study examined whether women wished to be screened again in a future pregnancy and confirmed unpublished findings that the majority do wish to be screened again.

Table 64 shows studies which assessed experiences of health professionals involved in the screening service. Our review identified some problems. It is apparent from some studies that the provision of information and counselling support is not always adequate and health professionals are often poorly informed. This will cause a degree of anxiety that could have been avoided. Correct information at the outset of the screening process is needed, together with sensitive communication of positive screening results by well-informed health professionals and the provision of appropriate support during the waiting period between the screen positive result and the diagnostic result.

An additional consideration in screening is that midwives are, in general, trained to respond to problems highlighted by the women and not by someone else (that is, the screener). This sometimes leads to resentment towards the screening test because the screeners may be viewed as an external party adding to their workload, and if midwives are poorly informed or inadequately trained they can perceive the screening test as an undesirable intrusion. This is exacerbated by the fact that the ultimate choice in the screening process is whether to have a termination of a pregnancy with Down's syndrome, an option that is considered with difficulty by some health professionals. Whatever the personal reservations or feelings of someone delivering the screening service may be, the test should be offered in a neutral manner, with its benefits, limitations, and consequences presented objectively.

Screening centres should hold training seminars where, as well as teaching about the theory of antenatal screening and keeping staff aware of local policy and screening results, the method of reporting screening results can be carefully taught. Choosing language to communicate screening results in a way that avoids creating unnecessary anxiety is difficult, and counselling workshops with role-play scenarios can be helpful in teaching this. It should also be recognised that it is the professional duty of staff delivering antenatal screening to keep informed, just as it is the duty of the screening centre to provide the opportunity for information and training.

If screening at 10 weeks of pregnancy were shown to be more effective and safe than screening later, it should be introduced provided that it is cost-effective. The necessary support should be at least as good as that offered at a later stage of pregnancy. Earlier screening would also require organisational changes in the timing of antenatal clinic or general practitioner visits. Also, in the urgency to conduct screening early in pregnancy, it is important that adequate time for choosing whether to be screened is given, and that women are not rushed into making a decision before they are ready to do so.

In screening centres all over the country, practical problems associated with antenatal screening are likely to recur and each screening centre is then bound to spend time trying to find the best solution. Consideration should be given to a national collection centre of screening problems or complaints. In this way, an anonymised form outlining the problem could be sent from the local screening centre to the national centre and a coordinator there, who would draw on a much wider experience, could suggest ways of putting the problem right. It would also provide a support structure for health professionals involved in antenatal screening, allowing them to share problems, and in doing so, help to deal with them.

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TABLE 64 *Serum screening for Down's syndrome: a review of studies on experiences of health professionals*

Study	Screening test	Study participants	Methods	Results	Main conclusions
Marteau *et al*, 1992[21]	AFP for DS and NTDs	53 midwives, 49 doctors in large London teaching hospital	Taped consultations with women booking for antenatal care at about 12 weeks of pregnancy. DS screening offered only to women ≥ 32 years (28 consultations)	AFP test mentioned in 84/102 consultations; DS mentioned in 4/28 consultations with women ≥ 32 years; likelihood of screen positive result never mentioned; meaning of screen positive result given in 10/64 consultations; meaning of screen negative result given in 2/64 consultations; procedure after screen positive result given in 28/84 consultations	Insufficient provision of information by health professionals on the meaning of screening results and their implications; screening presented in a way that encouraged women to undergo the test
Khalid *et al*, 1994[22]	DS serum screening; policy N/S	Anonymous questionnaire to 342 Leicestershire midwives; 188 (55%) completed questionnaires	Questionnaire included questions on grade, background training, attitudes to testing for DS and spina bifida, opinions on termination of pregnancy for foetal abnormality	40% of midwives did not feel confident when counselling for DS during serum screening; 38% did not feel termination was justified for DS; 25% were not in favour of the test	Midwives unprepared and experienced ethical dilemmas about screening
Green, 1994[23]	Serum screening; different policies	Questionnaire to 555 obstetricians in England and Wales	393 (71%) responded; 351 of these analysed. Questionnaires asked for DS screening policy in use and problems encountered	Of 315 obstetricians offering some form of screening, 146 felt resources insufficient to provide adequate counselling; 255/303 (84%) said most common problem was anxiety after FPR. 199/296 (67%) thought the offer of a test created anxiety. 245/301 (81%) thought women's understanding of the test was a problem. 151/300 (50%) thought midwives' understanding of the test was a problem	Inadequate provision of information and counselling to women, possibly owing to lack of resources
Smith *et al*, 1995[24]	DS serum screening; policy N/S	24/29 obstetricians and 63/97 midwives from six UK antenatal clinics agreed to participate; allocated to one of three groups: (1) received video-based training session and feedback; (2) received video-based training session; (3) controls. Intervention groups given information leaflet and pocket prompt card	Taped consultations with women booking for antenatal care. Knowledge measured by an MCQ of 19 questions about screening tests and population risks of genetic disorders; assessed at three study points: baseline, after training, and 3 months later. Measured on information-giving, communication skills, and knowledge of antenatal screening	35 full study participants. Information-giving and communication skills improved markedly in those receiving training and feedback on their performance, with the greatest improvement before feedback was given; those receiving only training without feedback greatly improved their communication skills and showed some improvement in information-giving	Modest improvements in communication could have been made with relatively brief training; greater improvements may be found if all staff are trained regularly
Sadler, 1997[25]	DS serum screening; double test	288 general practitioners, 200 midwives, 29 obstetricians in Portsmouth and South East Hants Health District	MCQ to assess professional knowledge about serum screening	84% response rate. Only 11% of health professionals correctly answered half of the factual questions about serum screening. Questions relating to sensitivity, specificity, and positive predictive value were particularly poorly answered. Obstetricians scored most highly; general practitioners scored significantly lower than the other groups	Women were not always given adequate information about the test owing to poorly informed health professionals. Training is needed

DS = Down's syndrome; N/S = not specified; MCQ = multiple choice questionnaire.

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Chapter 11

Quality assurance and monitoring

It is recognised that quality assurance and monitoring are important. Choice of suitable reagents and interpretive software is a first step, and, once a programme is under way, regular monitoring is needed. Shortcomings in screening may not be apparent for many months or years unless the process is monitored regularly. Well-run screening programmes use assay and epidemiological monitoring. Regular reviews should be conducted to determine whether the information and counselling needs of the service are being met.

Many centres have found that immediate responsibility for monitoring the programme should lie with the screening coordinator, who makes regular reports to the consultant in overall charge of the service, and keeps the staff delivering the service informed of its performance. The consultant in charge and the screening coordinator should have the authority to revise the screening programme, in consultation with colleagues as necessary, although in practice a weakness in some programmes is the lack of an overall authority over strategy and practice.

Choice of assay reagents and interpretive software

Assay reagents

The biochemical assays are usually performed using commercial diagnostic 'kits'. Assays should be shown to be accurate in that they yield the expected results in dilution and recovery experiments.

The following are the main points to be considered when choosing an assay kit.

1. Operating range – there should be a suitable working range of the assay; expected values for the samples should fall well within the standard range.
2. Accuracy – correct results should be obtained for samples with added analyte and with known definitions.
3. Precision – the precision of the assay should be satisfactory – that is, the interassay variation should be within acceptable limits, usually with a coefficient of variation of 10% or less. Assays can then be performed in singleton rather than duplicate, with savings in costs.
4. The assay should be easily performed in batches and results should be available within an acceptable period, usually not longer than 24 hours.
5. The performance of the assay should be acceptable, as assessed by an external quality assurance scheme (EQAS).
6. The assay should be cost-effective.

Interpretive software

Risk screening requires software for computer-assisted test interpretation. The following are the main points to be considered when choosing a software package.

1. The method of calculating risk should be established with the use of a published and validated algorithm based on statistical parameters that are available from the published scientific literature. It should not be a 'black box', the results of which cannot be independently checked. The software should have the ability to adjust serum marker levels for maternal weight, ethnic origin, diabetic status, and history of a previous affected pregnancy – all of which affect serum levels.
2. The software should be able to monitor assay levels and identify shifts from the expected (that is, the median levels should be, on average, 1.0 MoM), allowing users to interpret this and make adjustments as necessary.
3. It should be able to monitor detection rates, false-positive rates, and the odds of being affected given a positive result by comparing observed results with those expected in the screened population. A rise in the false-positive rate has implications for the number of women being referred for amniocentesis (namely, the financial cost and risk of miscarriage).
4. It should have a data tracking facility so that previous reports, revisions, and corrections can be reviewed at any time for the same woman during the pregnancy.
5. It should be user-friendly, enabling those involved in screening to implement the screening test and process the results quickly and efficiently.

Laboratory quality control

Internal quality control

Internal quality control involves developing criteria within the laboratory to ensure that assay results meet specified tolerance limits. Assay precision and accuracy are monitored, together with long- and short-term assay drift. Control serum samples for each serum marker span the range of values expected, and typically three controls (low, medium, and high) are used. Quality control pools usually last at least 12 months and preferably longer, so that long-term trends in assay performance can be established as well as day-to-day variation.

External quality control

EQASs serve different purposes from internal quality control measures. They are not designed to be applied to all assay runs, nor can they yield results quickly enough to allow decisions to be made about the acceptability of individual assays. EQASs are therefore not a substitute for internal quality control. They can, however, provide the following:

- an objective measure of individual laboratory and kit performance
- a mechanism for improving performance through knowledge and performance of other laboratories
- a vehicle for communicating and exchanging information with the EQAS organisers
- a resource centre for information
- reports to kit users of problems with test kits.

EQASs for serum screening operate by assessing the ability of laboratories to measure the serum markers reliably by distribution of samples which simulate actual clinical specimens. The laboratory's ability to convert concentrations of a serum marker in mass or international units into MoM values using its own reference data and to make screening interpretations is also evaluated, together with calculation of risk. Two examples of such schemes are the UK National External Quality Assurance Scheme (NEQAS), based in Edinburgh, and the scheme organised jointly by the Foundation for Blood Research in Maine, USA, and the American College of Pathologists in Chicago.

Epidemiological monitoring

Epidemiological monitoring is a quality control procedure described in Wald and Cuckle, 1980.[1] It uses data from the screened population, as well as the assay, to monitor the performance of the screening programme. It involves monitoring the following.

Medians

Observed median MoM values are examined for consistently high or low values (median MoM consistently above or below 1.0 MoM) and trends from high to low or vice versa (for example, too low in early gestation and too high in later gestation). If the medians in use are incorrect, this will affect the performance of the test. The rate of screen positives will be shifted upwards or downwards, and this will either increase the false-positive rate or decrease the detection rate, depending on the marker and direction of error.

False-positive rate

The false-positive rate can be monitored by examining the screen positive rate, because very few screen positives will prove to be affected pregnancies (about 1–2% of screen positives or 0.1% of all pregnancies screened) and there is no need to wait for the pregnancy outcome to monitor the rate effectively. The screen positive rate will be influenced by changes in the medians of the serum markers, the maternal age distribution of the screened population and, of course, the serum markers and risk cut-off level used in the programme.

Maternal age distribution of the screened population

At a given risk cut-off level the screen positive rate will be higher in older women than in younger women and, therefore, the false-positive and detection rates will also be higher. The age distribution of maternities has changed in Britain over time, with a greater proportion of births now occurring in older women. There are also differences in the maternal age distribution across the country. A recent report[2] has examined the effect on serum screening performance of variation in the age distribution of maternities between 1970 and 1993 in England and Wales, and between different health districts in 1991. In 1993, 9.2% of all maternities occurred in women aged ≥ 35 years compared with 7% in 1970. This percentage varied from 5% to 20% in health districts in 1991. The changes over time do not have a material effect on the performance of Down's syndrome screening, whereas the differences in age distributions between health districts do influence screening performance. If the triple test and a risk cut-off of 1 in 250 had been used, the detection rates would have varied from about 55% to 70% and the false-positive rate from 4.4% to 8.8% across different health districts *(Table 65)*. Differences in the age at which women have their pregnancies in different

TABLE 65 *Antenatal screening for Down's syndrome: expected screening performance according to the risk cut-off level in the district health authority with the largest percentage (20.1%) of maternities aged ≥ 35 years (Richmond, Twickenham, and Roehampton) and the district health authority with the smallest percentage (4.6%) of maternities aged ≥ 35 years (Hartlepool) in 1991*

Risk* cut-off level	DR (%)		FPR (%)		OAPR	
	Hartlepool	Richmond, Twickenham, and Roehampton	Hartlepool	Richmond, Twickenham, and Roehampton	Hartlepool	Richmond, Twickenham, and Roehampton
Double test†						
1:200	46	64	3.6	7.7	1:69	1:58
1:250	51	68	4.6	9.6	1:81	1:68
1:300	56	72	6.1	12.3	1:98	1:81
Triple test‡						
1:200	51	66	3.3	7.1	1:59	1:52
1:250	55	70	4.4	8.8	1:71	1:61
1:300	58	73	5.4	10.6	1:83	1:70

* *Risk of having a Down's syndrome live birth in the absence of prenatal screening and selective abortion, based on maternal age and serum markers.*
† *AFP and total hCG;* ‡ *AFP, uE_3, and total hCG measurements adjusted for maternal weight with gestational age estimated by dates.*

parts of the country mean that twice as many women may be referred for amniocentesis in some districts as in others when offered the same method of serum screening at the same risk cut-off level. The advantage of this approach is that it is equitable, but it does mean that the cost of the service varies according to local need.

Detection rate

An estimate of the detection rate will invariably be based on small numbers of observed Down's syndrome pregnancies in a screened population and the confidence limits on the detection rate will, therefore, be wide.

Ascertainment of all Down's syndrome pregnancies can be made by obtaining the outcomes of all pregnancies screened. This can be used to compare the predicted risk with the observed prevalence of Down's syndrome to validate the screening method. The observed number of cases of Down's syndrome can then be compared with the expected number, based on the age distribution of the screened population, to determine whether ascertainment is likely to have been complete.[3]

Uptake of screening and antenatal diagnosis

The effectiveness of an antenatal screening programme is likely to be judged by its effect on the birth prevalence of Down's syndrome. In this case it is not sufficient for the screening performance alone to be good; a reasonable number of women must choose to be screened in the first place, accept the offer of antenatal diagnosis if they are screen positive, and decide to terminate the pregnancy if found to be affected. Monitoring the rates of uptake of screening, antenatal diagnosis, and termination of affected pregnancies is therefore useful.

Ultrasound

With the advent of nuchal translucency measurement as a method of screening, there is a need to establish principles and criteria of quality control in much the same way as these were developed with the introduction of biochemical screening. Epidemiological monitoring in this respect need be, in principle, no different from such monitoring using biochemical measurements. In addition, it is probably worthwhile keeping a record of at least one ultrasound image per examination because this would encourage attention to quality, with the ultrasonographer likely to take more care if he or she is aware that the image can be re-examined at a later date.

Audit of service

Screening programmes must be delivered in a structured way with predictable performance, safety, and adequate information for all women, with an efficient system for handling the chain of events that form part of the whole screening process. Short audits of women's understanding and experience of the screening test are useful in assessing any problems. It is also helpful to hold regular meetings for clinical, midwifery and laboratory staff.

Conclusion

It is currently not possible to tell whether individual screening centres carry out appropriate monitoring procedures. The monitoring of laboratory and epidemiological data is important in ensuring an effective screening programme for Down's syndrome. NEQAS, which already monitors the laboratory aspects, attempted last year to collect epidemiological monitoring data (for example, screen positive rates and detection rates). It would be desirable to have such a national scheme in which both laboratory and epidemiological data can be reviewed regularly and systematically.

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Chapter 12

Achievements in Down's syndrome screening

Advances in efficacy of screening

Twenty years ago the only method of screening for Down's syndrome was to identify women of advanced maternal age and offer them an amniocentesis. This method of screening, while useful, was relatively ineffective, as it could detect no more than about 30% of affected pregnancies at the cost of carrying out an amniocentesis in 5% of all pregnant women. With the discovery of several biochemical markers associated with foetal Down's syndrome and the development of appropriate statistical methodology, multiple marker 'risk screening' can now detect up to 76% of affected pregnancies without increasing the proportion of women requiring an amniocentesis.

Introduction into medical practice

Antenatal screening for Down's syndrome using serum markers has become widespread. *Table 66* summarises screening in Britain where, in 1994, 60% of all pregnant women were offered some form of serum screening. *Table 67* summarises screening in the USA where, in 1995, about 60% of all pregnant women were screened using serum tests.

Screening provides couples with the opportunity to have an antenatal diagnosis and termination of an affected pregnancy if they so choose. It is not the intention of a screening programme that all affected pregnancies should be terminated unless all couples who have affected pregnancies wish to have a termination. In practice, not everyone will wish to be screened, and only a proportion of those

TABLE 67 *Screening for Down's syndrome in the USA, 1995*

Method of screening	Number of prenatal laboratories (%)	Number of women (%)
None	10 (4)	21,000 (1)
Maternal age with:		
AFP	29 (11)	532,000 (21)
AFP, hCG	35 (13)	
AFP, uE_3, hCG	175 (67)	1,945,000 (78)
Other combinations	14 (5)	
All serum	253 (96)	2,477,000 (99)
All	263 (100)	2,498,000* (100)

* *This represents about 60% of all annual births in the USA.*
Source: Palomaki et al.[2]

TABLE 66 *Screening for Down's syndrome in Britain in health districts and boards 1991–94*

Method of screening	Number (%) of health districts and boards			
	1991	1992	1993	1994
All women offered serum testing*				
AFP	28 (21)	17 (13)	9 (7)	6 (4)
Double test†	19 (14)	32 (24)	51 (38)	65 (49)
Triple test‡	15 (11)	11 (8)	11 (8)	10 (7)
Total	62 (46)	60 (45)	71 (53)	81 (60)
Maternal age only	65 (49)	50 (37)	37 (28)	31 (23)
Serum testing only to women above specified age	7 (5)	24 (18)	25 (19)	21 (16)
Nuchal translucency	–	–	1 (1)	1 (1)
All methods	134 (100)	134 (100)	134 (100)	134 (100)

* *All with maternal age.*
† *AFP and either total hCG or free β-hCG.*
‡ *AFP, uE_3 and either total or free β-hCG.*
Source: Wald et al, *1996.*[1] *Reproduced with permission of The Lancet Ltd.*

with positive screening results will choose to have an amniocentesis (about 80%). Even among those found to have a pregnancy with Down's syndrome, about 10% choose to continue with their pregnancy (see chapter 5). This means that even if serum screening for Down's syndrome was available to all women, the maximum expected reduction in the birth prevalence in the population would be about 50% (about 80% (screening uptake) × 75% (serum screening detection rate) × 90% (amniocentesis uptake in affected pregnancies) × 90% (uptake of termination of pregnancy)). This represents a reasonable expectation based on the performance of screening and parents' choices.

Acceptability of screening

Surveys of Down's syndrome screening have shown that serum screening is both feasible and acceptable and, although improvements in the quality of information and the provision of counselling are needed, overall there is evidence that a screening programme itself does not lead to lasting psychological harm (see chapter 10).

Validation of risk screening

The method of risk screening used in identifying affected pregnancies has been validated and found to be accurate.[3] This means that a reported (or predicted) term risk, based on maternal age and serum markers, is close to the observed birth prevalence. *Figure 27* shows the reported risk and observed prevalence based on about 76,000 pregnancies screened at Bart's[3] using the triple test (maternal age with AFP, uE_3, and total hCG); there is good agreement. *Figure 28* shows, similarly, data on about 20,000 pregnancies using the quadruple test (maternal age with AFP, uE_3, free α- and free β-hCG), again showing good agreement. There had been concern that risks reported to women during their pregnancy might not be accurate,[4] and so it is reassuring that they are. Risks are of clinical importance as they are used by the women and health professionals to make decisions. There is evidence that screen positive women with higher risks (say 1 in 10) are more likely to accept the offer of an amniocentesis than those with lower risks (say 1 in 200).[5,6]

Reduction in the birth prevalence of Down's syndrome

Figure 29 shows the effect of screening on the observed number of Down's syndrome live births, expressed as a percentage of the number of expected live births. In 1989, affected pregnancies were mainly identified using either maternal age or serum AFP or both. By 1994, multiple serum marker screening was established, and there has been a significant decline in the number of affected live births as more women are screened.

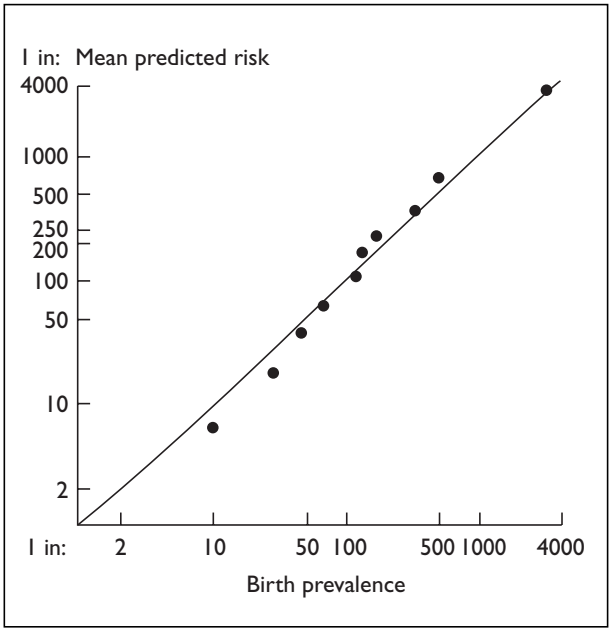

FIGURE 27 *Screening for Down's syndrome using the triple test: comparison of the predicted (reported) risk at term with the prevalence of Down's syndrome at term according to deciles of affected cases. The diagonal line represents perfect agreement*

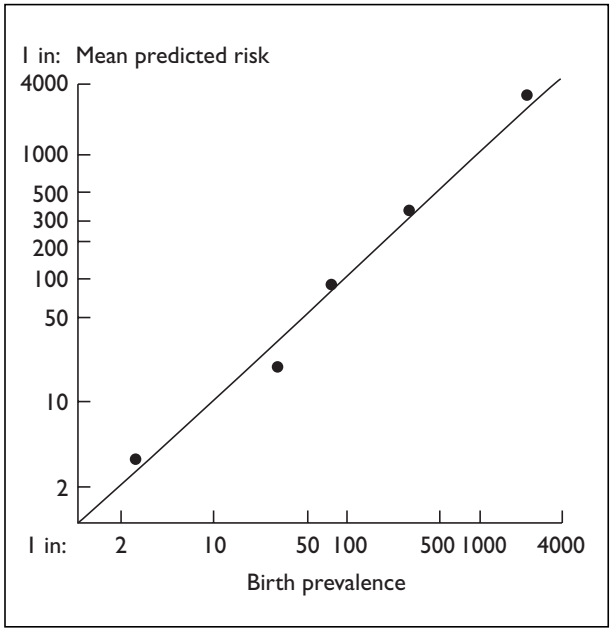

FIGURE 28 *Screening for Down's syndrome using the quadruple test: comparison of the predicted (reported) risk at term with the prevalence of Down's syndrome at term according to quintiles of affected cases. The diagonal line represents perfect agreement*

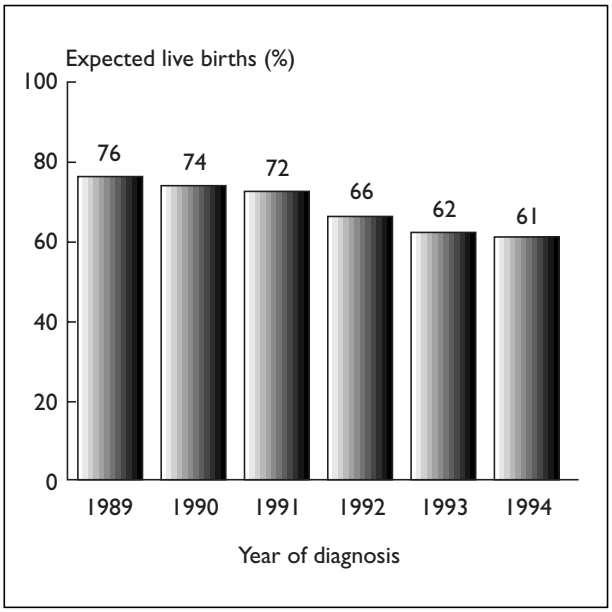

FIGURE 29 *Observed Down's syndrome live births in Britain expressed as a percentage of expected live births between 1989 and 1994 (Data from personal communication Morris J, Mutton D, National Down's Syndrome Cytogenetics Register)*

Research and development

Screening for Down's syndrome has become a widespread area of research as well as a service activity. There have been continuing improvements in the performance of serum screening and the provision of this service to women. It has brought together many areas of medicine, such as epidemiology, biochemistry, obstetrics and radiology. The discovery of first trimester ultrasound and serum markers holds the promise of more effective screening.

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Chapter 13

Current screening practice: problems and proposed solutions

Incomplete coverage and inconsistent practice

Screening in Britain is fragmented and incomplete. A survey of Down's syndrome screening in Britain[1] showed that, in 1994, one-third of health districts did not provide a serum screening service. Those that did so offered different combinations of serum markers. Some districts restricted serum testing to older women and a few districts started to implement ultrasound scanning as the method of screening. In some cases the same woman was offered screening at various stages in pregnancy; at about 10 weeks using nuchal translucency, at about 4–5 weeks later based on serum screening, and sometimes a few weeks later based on a further ultrasound examination. The situation is unsatisfactory. Both the public and the profession are confused. There is uncertainty over the best strategy and its expected screening performance.

Inequity and lack of access

Equal access is an important aspect of a screening service. All individuals who stand to gain from screening should have equal access to non-invasive tests which have proven efficacy. An appropriate screening policy is one which is designed to ensure that people of similarly high risk are offered further diagnostic tests. Current screening practice in Britain is inequitable, with access to established screening tests dependent on area of residence and, in some areas, a woman's age.

Problems with existing screening programmes

First, the administrative arrangements for screening vary across Britain. Sometimes these are based around the laboratory performing the assays, sometimes around an individual maternity unit, and sometimes administration is set up regionally covering several maternity units, laboratories, or both. There is often no clear line of responsibility and no clear mechanism for modifying screening policy, its delivery, or how the programme is monitored. Problems therefore appear with no system to deal with them and, as a consequence, progress can be difficult. For example, there may be no one person responsible for keeping abreast of screening developments and initiating the addition of new improved markers.

Secondly, single maternity units are too small to acquire adequate operational experience or monitor the service. For example, a maternity unit with 3000 deliveries per year might expect about four Down's syndrome births in 1 year. If only three of the affected pregnancies were screened and only one of these was detected, the confidence limits on the detection rate of the test would be 1–91%, too large to tell if there was a problem with the screening programme. It could take 4 years to gain sufficient numbers to make a valid judgment.

Finally, there is no national network of centres offering antenatal screening, resulting in variable levels of service throughout the country. Screening would benefit from the interaction of the professionals involved, promoting the dissemination of information and using the experience of others.

Stepwise screening

In some places a method of screening has emerged in which the decision to offer a woman serum testing depends on her age. Although not generally seen as such, it is a method of stepwise (or two-step) screening which involves administering more than one screening test for the same disorder to the same person in sequence. In this case, maternal age is, in effect, the first screening test and serum markers the second test. This approach is inefficient when compared with screening using the tests simultaneously because it increases the false-positive rate for a given detection rate (or it decreases the detection rate for a given false-positive rate). This means that a two-step screening programme is less safe, as there are more unaffected foetal losses associated with the invasive diagnostic procedure.[2]

There are two types of two-step screening.

Two-step screening: further screening of screen positives

Here, the first screening test is determining maternal age; women above a specified age, say 30 (that is, screen positive) are offered serum screening – that is, further screening of screen positives. Those who have positive results after the serum test are then offered a diagnostic test. This approach is carried out in a few districts in Britain. *Figure 30* (A) illustrates the screening performance using the triple test as the serum test; the overall detection rate is 46% for a 2.7% false-positive rate. *Figure 30* (B) shows the one-step equivalent in which all women, regardless of age, are offered a serum test and the detection rate is fixed at 46% as in (A), to allow a comparison of like with like. The false-positive rate is 1.0%. There would be 63% fewer false-positives (2.7 vs. 1.0%), 63% fewer amniocenteses, and 63% fewer unaffected foetuses lost owing to amniocentesis. The two-step approach has been adopted in some centres because it is less expensive (£13,000 per Down's syndrome birth avoided compared with £23,000 with the one-step equivalent), despite being less safe (more unaffected foetuses lost for a given detection rate) *(Table 68)*.

Two-step screening: further screening of screen negatives

The first screening test is again maternal age, but only women aged below a specified age, say 35 (that is, screen negative) are offered serum testing – further screening of screen negatives. All women above 35 years (that is, screen positive) are offered a diagnostic test

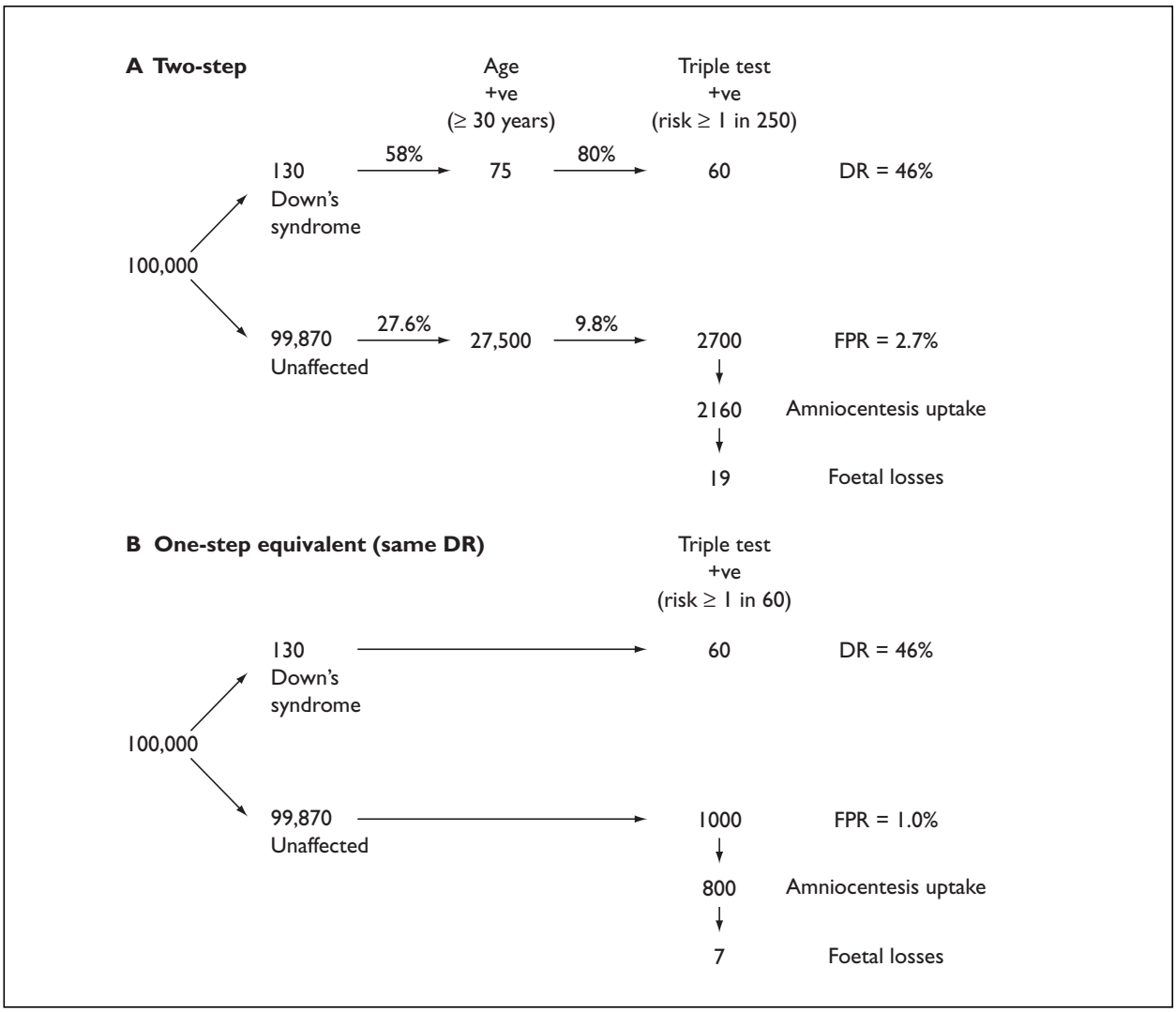

FIGURE 30 *Safety of two-step and one-step screening. (A) Two-step screening (further screening of screen positives) using the triple test (AFP, uE_3 and total hCG) with gestation by scan and allowance for maternal weight. Women are screen positive on the first test (maternal age) if they are aged ≥ 30 years and positive on the second test if their triple test risk is ≥ in 250. The uptake of amniocentesis is 80% and the unaffected foetal loss rate is 0.9%. (B) One-step equivalent to give the same DR as in (A)*

TABLE 68 *Comparison of one-step or stepwise screening for Down's syndrome using the triple test (AFP, uE_3, total hCG)*[*]

Screening policy	Risk cut-off level	Overall DR (%)	Overall FPR (%)	Unaffected foetal losses per Down's syndrome birth avoided	Cost (£) per Down's syndrome birth avoided
One-step screening					
Triple test for all women	1 in 250	69	4.9	0.49	22,000
Two-step screening					
(i) Rescreen positives: triple test for women aged ≥ 30	1 in 250	46	2.7	0.39	13,000
One-step equivalent to achieve same DR	1 in 60	46	1.0	0.14	23,000
(ii) Rescreen negatives: amniocentesis for women aged ≥ 35 and triple test for women < 35	1 in 250	73	11.4	0.11	32,000
One-step equivalent to achieve same DR	1 in 320	73	6.4	0.60	24,000

[*] *Gestation estimated by scan and marker levels corrected for maternal weight; samples assayed in singleton. Parameters used in the analysis are (a) Down's syndrome birth prevalence of 1.3 per 1000 (1.69 per 1000 in second trimester); (b) amniocentesis: 80% uptake in unaffected pregnancies and 90% uptake in affected pregnancies which are serum screened. (In (b) the uptake in those affected pregnancies who are offered an amniocentesis and not a serum test (that is, because they are aged ≥ 35) is taken to be the same as unaffected pregnancies (80%).) Cost of £150 (20% decline, cost of counselling £10); (c) termination of pregnancy: 90% uptake, cost £475; (d) procedure-related foetal loss rate: 0.9%, cost £475; (e) cost of triple test £9.60.*

without serum testing. This approach, illustrated in *Figure 31* (A), is widely used in the USA. The overall detection rate is 73% and the false-positive rate is 11.4%.

The one-step equivalent *(Figure 31* (B)*)*, in which the detection rate also is set at 73%, would yield a false-positive rate of 6.4%. There would be 44% fewer false-positives (11.4 vs. 6.4%), 44% fewer amniocenteses, and 44% fewer induced foetal losses. The two-step approach, in which older women are directly offered an amniocentesis and younger women a serum test, has been adopted because of an uncritical adherence to historical screening practice (age-only screening), even though it is more expensive and less safe *(Table 68)*. Two-step screening is always less effective than its one-step equivalent. One-step screening maintains the advantage of risk screening in that it maximises the detection rate for a given false-positive rate.

The ultrasound anomaly scan used to revise the screening risk

Although not often perceived as such, a variation of two-step screening of the 'further screening of positives' type is the practice in which women with screen positive serum results are offered a detailed 18–20 week ultrasound anomaly scan (which is, in effect, a second screening test) in order to revise their risk of having an affected pregnancy. In the absence of ultrasound markers indicative of Down's syndrome, (for example, nuchal fold thickening and reduced femur length, see chapter 6), a woman's risk based on the serum test is lowered, and there will be a tendency for her to be reassured and not undergo an invasive diagnostic test. The effect of this could be that about half the originally screen positive pregnancies with Down's syndrome would be missed, assuming that the anomaly scan has a detection rate of 50% for a 5% false-positive rate. This is illustrated in *Figure 32*, in which women with a risk of 1 in 200 based on the serum test would have their risk halved to 1 in 400 if the anomaly scan was negative. This practice is unsatisfactory because it will tend to give false reassurance to a woman who was screen positive on the first test, and became screen negative after the second test, but proceeds to have an affected birth. As well as being inefficient, this adds to the emotional stress of the woman. A diagnostic test should be offered to all women with a positive result after the serum test, regardless of a subsequent ultrasound scan result.

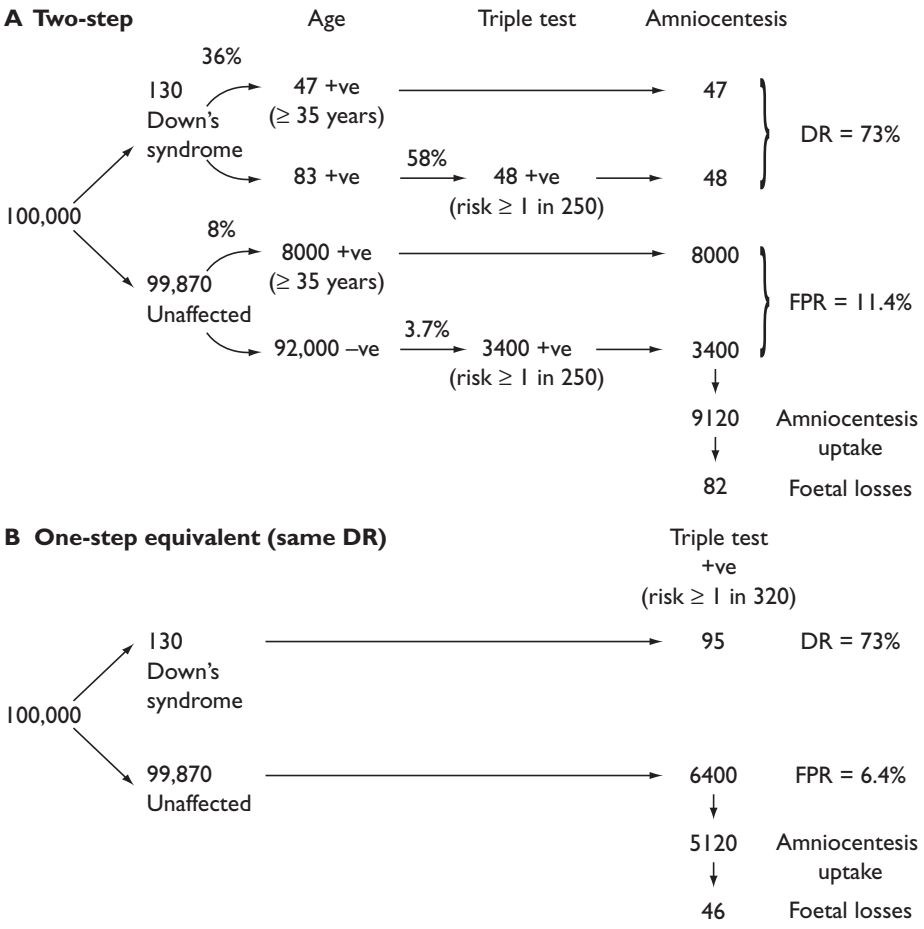

FIGURE 31 *Safety of two-step and one-step screening. (A) Two-step screening (diagnose positives, rescreen negatives) using the triple test (AFP, uE_3 and total hCG) with gestation by scan and allowance for maternal weight. Women are screen positive on the first test (maternal age) if they are aged ≥ 35 and then offered an amniocentesis. Screen negative women (aged ≤ 35) are offered serum screening and are positive if the triple test risk is ≥ in 250. The uptake of amniocentesis is 80% and the unaffected foetal loss rate is 0.9%. (B) One-step equivalent to give the same DR as in (A)*

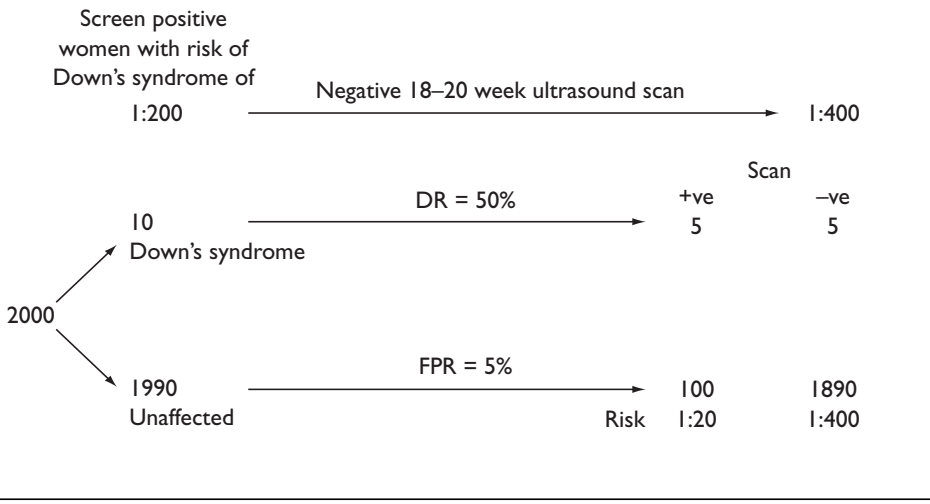

FIGURE 32 *Variation of two-step screening of the 'rescreen positives' type where an anomaly scan is used in women with positive serum screening results to alter the triple test risk. It is assumed that the anomaly scan has a DR of 50% and an FPR of 5%*

Premature introduction of ultrasound screening

Screening using ultrasound has been introduced into practice with little formal evidence. This is partly because ultrasound is used in the clinical management of patients and its extension to screening is less controllable than the introduction of a new serum screening service. Serum screening requires the explicit agreement of various groups within the screening team, an identifiable allocation of new resources, and is less easily absorbed into existing practice. The extension of ultrasound from a clinical activity to a screening activity tends to be subtle, and the increase in medical staff requirements tends to arise without explicitly linking the extra work to the increased screening activity.

A number of centres have introduced nuchal translucency measurement as the principal measurement of screening at 10–14 weeks. While it is clear that this is an effective marker for Down's syndrome, this review has shown that there is at present insufficient evidence to show that the performance of such screening is better than second trimester serum screening (see chapter 7).

Need for improvement in staff education and training

Inquiries from women who have been screened and from screening centres indicate that health professionals delivering the screening service would benefit from further training in Down's syndrome screening. Inadequate information provided at the outset leads to excess anxiety for women with screen positive results, inappropriate reassurance after a screen negative result and, in some instances, women having the screening test who, if the full implications had been adequately explained, would have declined it.

Organisation of the screening service

The screening service should be organised using a structure that will avoid the problems currently experienced in practice. To achieve this, a screening programme should be run from a single centre, where the laboratory assays, interpretation and monitoring of results, training, and coordination of the programme are based. The number of pregnancies screened at each centre needs to be high enough to accumulate sufficient experience, while still being small enough for those involved in the screening service to have sufficient influence and personal commitment to the service they deliver.

To provide the necessary professional leadership, a screening consultant should have overall responsibility, with the authority to make policy decisions and establish a team including obstetric, laboratory, nursing, and public health expertise. Day-to-day management of the programme would be delegated to a screening coordinator, who would work closely with the screening consultant. Resources should be allocated to cover all costs associated with the delivery of the screening service so that resources are available to those responsible for the service. Each centre with such an organisational structure could be termed a 'screening unit' and may offer a screening service to several maternity units.

Table 69 shows the implication of having screening units of different sizes starting from 5000 pregnancies per unit (the size of a typical maternity hospital) to 50,000 (equivalent to about ten maternity hospitals working together). The table shows the number of women with positive screening results, the number who would have an amniocentesis, the number who have a positive karyotype, and the number who require termination of pregnancy, each stage requiring further counselling. The level of experience gained from a small size unit (for example, 5000 births per year) would be insufficient, whereas a unit of, for example, 25,000 births or more per year would, perhaps, be too large to be covered by a single screening coordinator – including too many hospitals over too wide an area, and probably with too great a workload. It follows that a screening unit which has about 20,000 births a year, with about 30 Down's syndrome births expected in the absence of screening, is likely to be the most appropriate, though of course some variation around this would be acceptable.

The proposed size of such a screening unit would be large enough to monitor adequately all aspects of the service, run effective management training programmes, and reduce the costs associated with laboratory assay and interpretation of results through more efficient uses of resources, while still preserving the personal contacts and local involvement that are important in offering a medical service of this kind.

TABLE 69 *Estimated workload for screening units of 5000 to 50,000 pregnancies per year in England and Wales*

		Number of pregnancies in screening unit per year*				
		5000	**15,000**	**20,000**	**25,000**	**50,000**
(i)	No. of screening units in England and Wales	130	43	33	26	13
(ii)	No. of maternity units with 5000 deliveries per year in each screening unit	1	3	4	5	10
(iii)	Expected no. of Down's syndrome pregnancies (births)†	9 (7)	27 (21)	36 (28)	45 (35)	91 (70)
(iv)	No. of women accepting screening (80%)	4000	12,000	16,000	20,000	40,000
(v)	No. of women with positive results and who require counselling‡	200 (4 per week)	600 (12 per week)	800 (15 per week)	1000 (19 per week)	2000 (38 per week)
(vi)	No. of women who accept amniocentesis and require further counselling#	160 (3 per week)	480 (9 per week)	640 (12 per week)	800 (15 per week)	1600 (31 per week)
(vii)	No. of women with positive diagnostic test (that is, affected pregnancy) and require further counselling¶	5	14	18	23	46
(viii)	No. of women who accept termination of affected pregnancy and require further counselling**	4	13	16	21	41

* *Based on total number of births of 650,000 in England and Wales.*[1]
† *Birth prevalence of 1.4 per 1000; prevalence at second trimester of 1.4/0.77 per 1000 (0.77 is the natural loss rate of affected foetuses, see chapter 9).*
‡ *(iv) × 5% (triple test FPR).*
\# *(v) × 80% (amniocentesis uptake).*
¶ *(iii) × 80% (screening uptake) × 70% (triple test DR) × 90% (amniocentesis rate).*
** *(vii) × 90% (uptake of termination of pregnancy).*

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Chapter 14

Recommendations

Organisation of screening services

There is substantial variation in screening services for Down's syndrome throughout Britain and there are centres screening too few pregnancies to obtain sufficient operational experience or to monitor the service adequately. This is unlikely to change materially with the current district-based administrative arrangements. There needs to be greater central direction over screening, with a written policy, specified funding, and line responsibility, while preserving the strength of local commitment to the service.

The authors recommend that:
- screening is organised from about 35 screening centres throughout the country, each including three to four maternity units with a total of about 15,000 births a year (about 30 with Down's syndrome). At each centre a screening consultant with overall responsibility for the service and a dedicated screening coordinator, who would work together with the consultant, should be appointed
- the screening consultants and coordinators would form a national network that would be responsible for ensuring an even and consistent service throughout the country and compile an annual report on screening for Down's syndrome in Britain
- the purchase of Down's syndrome screening services should be separated from the general obstetric budget so that each screening centre has dedicated resources and both the authority and responsibility needed to provide the specified screening service
- each screening centre should meet agreed criteria for the method of screening, provision of information and counselling services, and monitoring information.

Access and equity

It is unsatisfactory that some women do not have access to serum screening for Down's syndrome by virtue of their age or where they happen to live.

- The authors recommend that all pregnant women should have equal access to serum screening for Down's syndrome regardless of where they live or their age.

Avoidance of multistep screening

The tendency to offer more than one method of screening to the same women at different stages of pregnancy is confusing and inefficient. Examples of this unsatisfactory practice are serum testing offered to women above or below a certain age or a nuchal translucency examination offered at about 11 weeks followed by a serum screening test at about 15 weeks and, possibly, even a subsequent anomaly scan at about 18 weeks.

- The authors recommend that in the purchase of screening services, arrangements are made to ensure that screening is carried out at one stage in pregnancy and offered to all pregnant women.

Screening policy

While we recognise that there has been controversy over whether the double or triple test should be used in screening, the evidence indicates that screening using the triple test with maternal age is more effective, safer and, financially, more cost-effective than the use of the double test. Recently, the quadruple test including inhibin A appears to have somewhat better performance. Serum markers and nuchal translucency have been shown to be effective in screening for Down's syndrome in the first trimester.

The authors recommend that, at present:
- second trimester serum screening be the standard method of screening and that consideration be given to centres using either the triple or quadruple tests
- an ultrasound scan examination should not be used to revise a woman's risk after she has undergone serum screening
- first trimester screening (with serum and ultrasound markers) needs further evaluation before a decision is made to introduce it into general routine practice; this decision should rest on whether first trimester screening is shown

to be at least as effective, safe and financially cost-effective as second trimester screening
- carefully monitored pilot programmes of first trimester screening be conducted to determine the logistics of combining ultrasound and serum markers at centres with theoretical and practical expertise.

Education and training

There is evidence that better staff education and training is needed so that patients are adequately informed about screening and its implications and they have confidence in the care that they receive.

- The authors recommend that specialised training for health professionals involved in screening should be mandatory and conducted by regional centres experienced in Down's syndrome screening. This should include training in both the theoretical and practical aspects of the screening service.

Further research

It is recognised that further research will undoubtedly improve screening performance. An important area of research that is currently in progress is the evaluation of first trimester screening using a combination of serum markers and ultrasound. Other important areas include the study of urinary markers and foetal cells in maternal blood.

- The authors recommend that research, particularly collaborative multicentre research, should be encouraged at screening centres because of the need to accrue sufficient numbers of affected pregnancies for statistical reliability.

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Any errors of fact and interpretations from the data presented are the responsibility of the authors.

St Bartholomew's Hospital and the Royal London School of Medicine and Dentistry together with the Foundation for Blood Research and Women and Infants Hospital (Providence Rhode Island) hold a patent for uE_3 measurement as a marker for Down's syndrome screening. Professor Wald is a director of Logical Medical Systems Limited, which produces αlpha™, a commercial interpretive software package for Down's syndrome screening using ultrasound and serum markers.

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This report was identified as a priority by the Population Screening Panel.

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