First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

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[Diagnostic Test Accuracy Review]

First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

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ABSTRACT

Background

Down's syndrome occurs when a person has three copies of chromosome 21 (or the specific area of chromosome 21 implicated in causing Down's syndrome) rather than two. It is the commonest congenital cause of mental disability. Non-invasive screening based on biochemical analysis of maternal serum or urine, or fetal ultrasound measurements, allows estimates of the risk of a pregnancy being affected and provides information to guide decisions about definitive testing.

Before agreeing to screening tests, parents need to be fully informed about the risks, benefits and possible consequences of such a test. This includes subsequent choices for further tests they may face, and the implications of both false positive (i.e. invasive diagnostic testing, and the possibility that a miscarried fetus may be chromosomally normal) and false negative screening tests (i.e. a fetus with Down's syndrome will be missed). The decisions that may be faced by expectant parents inevitably engender a high level of anxiety at all stages of the screening process, and the outcomes of screening can be associated with considerable physical and psychological morbidity. No screening test can predict the severity of problems a person with Down's syndrome will have.

Objectives

To estimate and compare the accuracy of first and second trimester serum markers with and without first trimester ultrasound markers for the detection of Down's syndrome in the antenatal period, as combinations of markers.

Search methods

We conducted a sensitive and comprehensive literature search of MEDLINE (1980 to 25 August 2011), Embase (1980 to 25 August 2011), BIOSIS via EDINA (1985 to 25 August 2011), CINAHL via OVID (1982 to 25 August 2011), the Database of Abstracts of Reviews of Effectiveness (the Cochrane Library 25 August 2011), MEDION (25 August 2011), the Database of Systematic Reviews and Meta-Analyses in Laboratory Medicine (25 August 2011), the National Research Register (Archived 2007), and Health Services Research Projects in Progress database (25 August 2011). We did not apply a diagnostic test search filter. We did forward citation searching in ISI citation indices, Google Scholar and PubMed 'related articles'. We also searched reference lists of retrieved articles

Selection criteria

Studies evaluating tests of combining first and second trimester maternal serum markers in women up to 24 weeks of gestation for Down's syndrome, with or without first trimester ultrasound markers, compared with a reference standard, either chromosomal verification or macroscopic postnatal inspection.

Data collection and analysis

Data were extracted as test positive/test negative results for Down's and non-Down's pregnancies allowing estimation of detection rates (sensitivity) and false positive rates (1-specificity). We performed quality assessment according to QUADAS criteria. We used hierarchical summary ROC meta-analytical methods to analyse test performance and compare test accuracy. Analysis of studies allowing direct comparison between tests was undertaken. We investigated the impact of maternal age on test performance in subgroup analyses.

Main results

Twenty-two studies (reported in 25 publications) involving 228,615 pregnancies (including 1067 with Down's syndrome) were included. Studies were generally high quality, although differential verification was common with invasive testing of only high risk pregnancies. Ten studies made direct comparisons between tests. Thirty-two different test combinations were evaluated formed from combinations of eight different tests and maternal age; first trimester nuchal translucency (NT) and the serum markers AFP, uE3, total hCG, free βhCG, Inhibin A, PAPP-A and ADAM 12. We looked at tests combining first and second trimester markers with or without ultrasound as complete tests, and we also examined stepwise and contingent strategies.

Meta-analysis of the six most frequently evaluated test combinations showed that a test strategy involving maternal age and a combination of first trimester NT and PAPP-A, and second trimester total hCG, uE3, AFP and Inhibin A significantly outperformed other test combinations that involved only one serum marker or NT in the first trimester, detecting about nine out of every 10 Down's syndrome pregnancies at a 5% false positive rate. However, the evidence was limited in terms of the number of studies evaluating this strategy, and we therefore cannot recommend one single screening strategy.

Authors' conclusions

Tests involving first trimester ultrasound with first and second trimester serum markers in combination with maternal age are significantly better than those without ultrasound, or those evaluating first trimester ultrasound in combination with second trimester serum markers, without first trimester serum markers. We cannot make recommendations about a specific strategy on the basis of the small number of studies available.

PLAIN LANGUAGE SUMMARY

Screening tests for Down's syndrome in the first 24 weeks of pregnancy

Background

Down's syndrome (also known as Down's or Trisomy 21) is an incurable genetic disorder that causes significant physical and mental health problems, and disabilities. However, there is wide variation in how Down's affects people. Some individuals are severely affected whilst others have mild problems and are able to lead relatively normal lives. There is no way of predicting how badly a baby might be affected.

Expectant parents are given the choice to be tested for Down's syndrome during pregnancy to assist them in making decisions. If a mother is carrying a baby with Down's syndrome, then there is the decision about whether to terminate or continue with the pregnancy. The information offers parents the opportunity to plan for life with a child with Down's syndrome.

The most accurate tests for Down's syndrome involve testing fluid from around the baby (amniocentesis) or tissue from the placenta (chorionic villus sampling (CVS)) for the abnormal chromosomes associated with Down's syndrome. Both these tests involve inserting needles through the mother's abdomen and are known to increase the risk of miscarriage. Thus, the tests may not be suitable for all pregnant women. Rather, tests that measure markers in the mother's blood, urine, or on ultrasound scans of the baby are used for screening. These screening tests are not perfect as they can miss cases of Down's syndrome and also give high risk test results to a

number of women whose babies are not affected by Down's syndrome. Thus, pregnancies identified as high risk using these screening tests require further testing using amniocentesis or CVS to confirm a diagnosis of Down's syndrome.

What we did

We assessed combinations of first trimester (up to 14 weeks' gestation) and second trimester serum screening tests (up to 24 weeks' gestation), with or without first trimester ultrasound screening tests, Our aim was to identify the most accurate test(s) for predicting the risk of a pregnancy being affected by Down's syndrome. We looked at one ultrasound marker (nuchal translucency) and seven different serum markers (PAPP-A, total hCG, free βhCG, uE3, AFP, inhibin A, ADAM 12) that can be used alone, in ratios or in combination, taken before 24 weeks' gestation, thus creating 32 screening tests for Down's. We found 22 studies, involving 228,615 pregnancies (including 1067 fetuses affected by Down's syndrome).

What we found

For Down's syndrome screening, where tests were carried out in the first and second trimester and combined to give an overall risk, we found that a test comprised of first trimester nuchal translucency and PAPP-A, and second trimester total hCG, uE3, AFP and Inhibin A was the most sensitive test, detecting nine out of 10 pregnancies affected by Down's syndrome. Five per cent of pregnant women receiving a high risk test result based on this combination would not be affected by Down's syndrome. There were relatively few studies assessing these tests and therefore we cannot make a strong recommendation about the best test.

Other important information to consider

The ultrasound tests themselves have no adverse effects for the woman, and blood tests can cause discomfort, bruising and, rarely, infection. However, some women who have a high risk screening test result, and are given amniocentesis or CVS have a risk of miscarrying a baby unaffected by Down's. Parents will need to weigh up this risk when deciding whether or not to have an amniocentesis or CVS following a high risk screening test result.

BACKGROUND

This is one of a series of reviews on antenatal screening for Down's syndrome following a generic protocol (Alldred 2010) - see Published notes for more details.

Target condition being diagnosed

Down's syndrome

Down's syndrome affects approximately one in 800 live born babies (Cuckle 1987a). It results from a person having three, rather than two, copies of chromosome 21 - or the specific area of chromosome 21 implicated in causing Down's syndrome - as a result of trisomy (an additional copy of the whole chromosome) or translocation (duplication of part of the chromosome caused by rearrangements of parts of different chromosomes, resulting in three copies of information responsible for Down's syndrome). If not all cells are affected, the pattern is described as 'mosaic'. Down's syndrome can cause a wide range of physical and mental problems. It is the commonest cause of mental disability, and is also associated with a number of congenital malformations, notably affecting the heart. There is also an increased risk of cancers such as leukaemia, and numerous metabolic problems including diabetes and thyroid disease. Some of these problems may be life-threatening, or lead to considerable ill health, while some individuals with Down's syndrome have only mild problems and can lead a relatively normal life.

There is no cure for Down's syndrome, and antenatal diagnosis allows for preparation for the birth and subsequent care of a baby with Down's syndrome, or for the offer of a termination of pregnancy. Having a baby with Down's syndrome is likely to have a significant impact on family and social life, relationships and parents' work. Special provisions may need to be made for education and care of the child, as well as accommodating the possibility of periods of hospitalisation.

Definitive invasive tests (amniocentesis and chorionic villus sampling (CVS)) exist that allow the diagnosis of Down's syndrome before birth but carry a risk of miscarriage. No test can predict the severity of problems a person with Down's syndrome will have.

Non-invasive screening tests based on biochemical analysis of maternal serum or urine, or fetal ultrasound measurements, allow an estimate of the risk of a pregnancy being affected and provide parents with information to enable them to make choices about definitive testing. Such screening tests are used during the first and second trimester of pregnancy.

Screening tests for Down's syndrome

Initially, screening was determined solely by using maternal age to classify a pregnancy as high or low risk for trisomy 21, as it was known that older women had a higher chance of carrying a baby with Down's syndrome (Penrose 1933).

Further advances in screening were made in the early 1980s, when Merkatz and colleagues investigated the possibility that low maternal serum alpha-fetoprotein (AFP), obtained from maternal blood in the second trimester of pregnancy could be associated with chromosomal abnormalities in the fetus. Their retrospective case-control study showed a statistically significant relationship between fetal trisomy, such as Down's syndrome, and lowered maternal serum AFP (Merkatz 1984). This was further explored by Cuckle and colleagues in a larger retrospective trial using data collected as part of a neural tube defect (NTD) screening project (Cuckle 1984a). This work was followed by calculation of risk estimates using maternal serum AFP values and maternal age, which ultimately led to the introduction of the two screening parameters in combination (Alfirevic 2004).

In 1987, in a small case-control study of women carrying fetuses with known chromosomal abnormalities, Bogart and colleagues investigated maternal serum levels of human chorionic gonadotrophin (hCG) as a possible screening tool for chromosomal abnormalities in the second trimester (Bogart 1987). This followed the observations that low hCG levels were associated with miscarriages, which are commonly associated with fetal chromosomal abnormalities. They concluded that high hCG levels were associated with Down's syndrome and because hCG levels plateau at 18 to 24 weeks, that this would be the most appropriate time for screening. Later work suggested that the ß sub-unit of hCG (free βhCG) was a more effective marker than total hCG (Macri 1990; Macri 1993).

Second trimester unconjugated oestriol (uE3), produced by the fetal adrenals and the placenta, was also evaluated as a potential screening marker. In another retrospective case-control study, uE3 was shown to be lower in Down's syndrome pregnancies compared with unaffected pregnancies. When used in combination with AFP and maternal age, it appeared to identify more pregnancies affected by Down's syndrome than AFP and age alone (Canick 1988). Further work suggested that all three serum markers (AFP, hCG and uE3) showed even higher detection rates when combined with maternal age (Wald 1988a; Wald 1988b) and appeared to be a cost-effective screening strategy (Wald 1992a).

Three other serum markers, produced by the placenta, have been linked with Down's syndrome, namely pregnancy-associated plasma protein A or PAPP-A, inhibin A and a disintegrin and metalloprotease 12 (ADAM12). PAPP-A has been shown to be reduced in the first trimester of Down's syndrome pregnancies, with its most marked reduction in the early first trimester (Bersinger 1995). Inhibin A is high in the second trimester in pregnancies affected by Down's syndrome (Cuckle 1995; Wallace 1995). There are some issues concerning the biological stability - for example, delay in samples arriving in the laboratory - and hence reliability of this marker, and the effect this will have on individual risk. ADAM 12 has been shown to be a potential first trimester marker with reduced maternal serum levels in pregnancy prior to 10 weeks (Laigaard 2003; Spencer 2008a).

In 1992, Nicolaides and colleagues demonstrated an association between increased nuchal translucency (NT) and chromosomal abnormalities (Nicolaides 1992). Nuchal translucency measurement requires an ultrasound scan of the fluid at the fetal neck between 10 and 13+6 weeks' gestation. If the amount is large, it suggests an increased risk of Down's syndrome. This study was small (827 women), but led to further research into the use of NT scanning and its value when combined with serum tests. Other first trimester ultrasound markers, such as absent nasal bone, abnormal ductus venosus flow velocity and tricuspid regurgitation, have also been investigated.

In addition to serum and ultrasound markers for Down's syndrome, work has been carried out looking at urinary markers. These markers include invasive trophoblast antigen, ß-core fragment, free ßhCG and total hCG (Cole 1999). There is controversy about their value (Wald 2003a).

Screening and parental choice

Antenatal screening is used for several reasons (Alfirevic 2004), but the most important is to enable parental choice regarding pregnancy management and outcome. Before a woman and her partner opt to have a screening test, they need to be fully informed about the risks, benefits and possible consequences of such a test. This includes the choices they may have to face should the result show that the woman has a high risk of carrying a baby with Down's syndrome and the implications of both false positive and false negative screening tests. They need to be informed of the risk of a miscarriage due to invasive diagnostic testing, and the possibility that a miscarried fetus may be chromosomally normal. If, following invasive diagnostic testing, the fetus is shown to have Down's syndrome, further decisions need to be made about continuation or termination of the pregnancy, the possibility of adoption and finally, preparation for parenthood. Equally, if a woman has a test that shows she is at a low risk of carrying a fetus with Down's syndrome, it does not necessarily mean that the baby will be born with a normal chromosomal make up. This possibility can only be excluded by an invasive diagnostic test (Alfirevic 2003). The deci-

sions that may be faced by expectant parents inevitably engender a high level of anxiety at all stages of the screening process, and the outcomes of screening can be associated with considerable physical and psychological morbidity. No screening test can predict the severity of problems a person with Down's syndrome will have.

Index test(s)

This review examined serum screening tests used in the first and second trimester of pregnancy (up to 24 weeks' gestation) with and without first trimester ultrasound tests (up to 14 weeks' gestation). The review examined the following individual markers; NT measurement in the first trimester, ADAM 12, AFP, uE3, total hCG, free βhCG, Inhibin A and PAPP-A. These markers can be used individually, in combination with age, and can also be used in combination with each other. The risks are calculated by comparing a woman's test result for each marker with values for an unaffected population, and multiplying this with her age-related risk. Where several markers are combined, risks are computed using risk equations (often implemented in commercial software) that take into account the correlational relationships between the different markers and marker distributions in affected and unaffected populations.

Stepwise testing allows for triage of women into risk categories at two stages. Women found to be very high risk at the end of first trimester screening are offered invasive testing, whereas those women deemed to be lower risk are then screened again in the second trimester and a further overall risk is calculated once both stages of the test are completed.

Contingent screening is similar, however at the completion of first trimester screening women are classified into three groups - high risk, medium risk and low risk. High risk women are offered invasive testing at this stage, low risk women undergo no further screening and medium risk women are offered second trimester serum tests and calculation of a further overall risk once both stages of the test are completed.

Alternative test(s)

Down's syndrome can be detected during pregnancy with invasive diagnostic tests such as amniocentesis or CVS, with or without prior screening. The ability to determine fetal chromosomal make up (also known as a karyotype) from amniotic fluid samples was demonstrated in 1966 by Steele and Breg (Steele 1966), and the first antenatal diagnosis of Down's syndrome was made in 1968 (Valenti 1968). Amniocentesis is an invasive procedure which involves taking a small sample of the amniotic fluid (liquor) surrounding the baby, using a needle which goes through the abdominal wall into the uterus, and is usually performed after 15 weeks' gestation. CVS involves taking a sample of the placental tissue using a needle which goes through the abdominal wall and uterus or a cannula through the cervix. It is usually performed between 10 and 13 weeks' gestation. Amniocentesis and CVS are both methods of obtaining fetal chromosomes material, which are then used to diagnose Down's syndrome. Both tests use ultrasound scans to guide placement of the needle. Amniocentesis carries a risk of miscarriage in the order of 1%; transabdominal CVS may carry a similar risk (Alfirevic 2003). Recent developments in the use of cell-free fetal DNA detection in maternal serum are paving the way for non-invasive diagnosis of Down's syndrome and other trisomies, however these tests were not used as reference standards in any of the studies examined.

There are many different screening tests which are available and offered which are the subject of additional Cochrane reviews and there are other reviews looking at this area. Tests being assessed in the other Cochrane reviews include first trimester serum tests (Alldred 2015); urine tests (Alldred 2015a); second trimester serum markers (Alldred 2012); and first trimester ultrasound tests alone, or in combination with first trimester serum tests (in press). Second trimester ultrasound markers have been assessed in a previous systematic review (Smith-Bindman 2001).

Rationale

This is one of a suite of Cochrane reviews, the aim of which is to identify all screening tests for Down's syndrome used in clinical practice, or evaluated in the research setting, in order to try to identify the most accurate test(s) available, and to provide clinicians, policy makers and women with robust and balanced evidence on which to base decisions about interpreting test results and implementing screening policies to triage the use of invasive diagnostic testing. The full set of reviews is described in the generic protocol (Alldred 2010).

A systematic review of second trimester ultrasound markers for detection of Down's syndrome concluded that nuchal fold thickening may be useful in detecting Down's syndrome, but that it was not sensitive enough to be used as a screening test (Smith-Bindman 2001). The review concluded that other second trimester ultrasound markers did not usefully distinguish between Down's syndrome and pregnancies without Down's syndrome. There has been no systematic review and meta-analysis of serum, urine and first trimester ultrasound markers to enable rigorous and robust conclusions to be made about the diagnostic accuracy of available Down's syndrome screening tests.

The topic has been split into several different reviews to allow for greater ease of reading and greater accessibility of data, and also to allow the reader to focus on separate groups of tests, for example, first trimester serum tests alone, first trimester ultrasound alone, first trimester serum and ultrasound, second trimester serum alone, first and second trimester serum, combinations of serum and ultrasound markers and urine markers alone. An overview review will compare the best tests, focusing on commonly used strategies, from each of these groups to give comparative results between the

best tests in the different categories. This review is written with the global perspective in mind, rather than to conform with any specific local or national policy, as not all tests will be available in all areas where screening for Down's syndrome is carried out.

OBJECTIVES

The aim of this review was to estimate and compare the accuracy of first and second trimester serum markers with and without first trimester ultrasound markers for the detection of Down's syndrome in the antenatal period, as combinations of markers. Individual markers are described in the other reviews belonging to this suite. Accuracy is described by the proportion of fetuses with Down's syndrome detected by screening before birth (sensitivity or detection rate) and the proportion of women with a low risk (normal) screening test result who subsequently had a baby unaffected by Down's syndrome (specificity).

Investigation of sources of heterogeneity

We planned to investigate whether a uniform screening test is suitable for all women, or whether different screening methods are more applicable to different groups, defined by advanced maternal age, ethnic groups and aspects of the pregnancy and medical history such as multiple pregnancy, diabetes and family history of Down's syndrome. We also considered whether there existed evidence of overestimation of test accuracy in studies evaluating risk equations in the derivation sample rather than in a separate validation sample.

METHODS

Criteria for considering studies for this review

Types of studies

We included studies in which all women from a given population had one or more index test(s) compared to a reference standard. Both consecutive series and diagnostic case-control study designs were included. Randomised trials where individuals were randomised to different screening strategies and all verified using a reference standard were also eligible for inclusion. Studies in which test strategies were compared head-to-head either in the same women, or between randomised groups were identified for inclusion in separate comparisons of test strategies. Studies were excluded if they included less than five Down's syndrome cases, or more than 20% of participants were not followed up.

Participants

Pregnant women up to 24 weeks' gestation confirmed by ultrasound, who had not undergone previous testing for Down's syndrome in their pregnancy were eligible. Studies were included if the pregnant women were unselected, or if they represented groups with increased risk of Down's syndrome, or difficulty with conventional screening tests including maternal age greater than 35 years old, multiple pregnancy, diabetes mellitus and family history of Down's syndrome.

Index tests

The following index tests were examined; nuchal translucency (NT) scanning, ADAM12, AFP, uE3,total hCG, free βhCG, Inhibin A, PAPP-A, and combinations of these markers with maternal age. Combinations without maternal age were excluded.

We looked at comparisons of tests in isolation and in various combinations. All strategies included first and second trimester serum tests, and some included additional first trimester ultrasound markers. The maximum number of markers in any one test was seven, in combination with maternal age.

Where tests were used in comparison we looked at the performance of test comparisons according to predicted probabilities computed using risk equations and dichotomised into high risk and low risk (and medium risk, where applicable).

Target conditions

Down's syndrome in the fetus due to trisomy, translocation or mosaicism.

Reference standards

We considered several reference standards, involving chromosomal verification and postnatal macroscopic inspection.

Amniocentesis and chorionic villus sampling (CVS) are invasive chromosomal verification tests undertaken during pregnancy. They are highly accurate, but the process carries a 1% miscarriage rate, and therefore they are only used in pregnancies considered to be at high risk of Down's, or at the mother's request. All other types of testing (postnatal examination, postnatal karyotyping, birth registers and Down's syndrome registers) are based on information available at the end of pregnancy. The greatest concern is not their accuracy, but the loss of the pregnancy to miscarriage between the serum test and the reference standard. Miscarriage with cytogenetic testing of the fetus is included in the reference standard where available. We anticipated that older studies, and studies undertaken in older women are more likely to have used invasive chromosomal verification tests in all women.

Studies undertaken in younger women and more recent studies were likely to use differential verification as they often only used prenatal karyotypic testing on fetuses considered screen positive/

high risk according to the screening test; the reference standard for most unaffected infants being observing a phenotypically normal baby. Although the accuracy of this combined reference standard is considered high, it is methodologically a weaker approach as pregnancies that miscarry between the index test and birth are likely to be lost from the analysis, and miscarriage is more likely to occur in Down's than normal pregnancies. We investigated the impact of the likely missing false negative results in sensitivity analyses.

Search methods for identification of studies

We used one generic search strategy to identify studies for all reviews in this series.

Electronic searches

We applied a sensitive search strategy to search the following databases using the text words and MeSH terms detailed in Appendix 1, adapting the search strategy for each different database.

The following databases were searched.

1. MEDLINE via OVID (1980 to 25 August 2011)
2. Embase via Dialog Datastar (1980 to 25 August 2011)
3. BIOSIS via EDINA (1985 to 25 August 2011)
4. CINAHL via OVID (1982 to 25 August 2011)
5. The Database of Abstracts of Reviews of Effectiveness (25 August 2011)
6. MEDION (25 August 2011)
7. The Database of Systematic Reviews and Meta-Analyses in Laboratory Medicine (www.ifcc.org/) (25 August 2011)
8. The National Research Register (Archived 2007)
9. Health Services Research Projects in Progress database (HSRPROJ) (25 August 2011)

The search strategy combined three sets of search terms (see Appendix 1). The first set was made up of named tests, general terms used for screening/diagnostic tests and statistical terms. Note that the statistical terms were used to increase sensitivity and were not used as a methodological filter to increase specificity. The second set was made up of terms that encompass Down's syndrome and the third set made up of terms to limit the testing to pregnant women. All terms within each set were combined with the Boolean operator OR and then the three sets were combined using AND. The terms used were a combination of subject headings and free-text terms. The search strategy was adapted to suit each database searched.

We attempted to identify cumulative papers that reported data from the same data set, and contacted authors to obtain clarification of the overlap between data presented in these papers, in order to prevent data from the same women being analysed more than once.

Searching other resources

In addition, we examined references cited in studies identified as being potentially relevant, and those cited by previous reviews. We contacted authors of studies where further information was required.

We carried out forward citation searching of relevant items, using the search strategy in ISI citation indices, Google scholar and Pubmed 'related articles'.

We did not apply language restrictions to the search.

Data collection and analysis

Selection of studies

Two review authors screened the titles and abstracts (where available) of all studies identified by the search strategy. Full-text versions of studies identified as being potentially relevant were obtained and independently assessed by two review authors for inclusion, using a study eligibility screening pro forma according to the pre-specified inclusion criteria. Any disagreement between the two review authors was settled by consensus, or where necessary, by a third party.

Data extraction and management

A data extraction form was developed and piloted using a subset of 20 identified studies (from all identified studies in this suite of reviews). Two review authors independently extracted data, and where disagreement or uncertainty existed, a third review author validated the information extracted.

Data on each marker were extracted as binary test positive/test negative results for Down's and non-Down's pregnancies, with a high risk result - as defined by each individual study - being regarded as test positive (suggestive or diagnostic of Down's syndrome), and a low risk result being regarded as test negative (suggestive of absence of Down's syndrome). Where results were reported at several thresholds, we extracted data at each threshold.

We noted those in special groups that posed either increased risk of Down's syndrome or difficulty with conventional screening tests including maternal age greater than 35 years old, multiple pregnancy, diabetes mellitus and family history of Down's syndrome.

Assessment of methodological quality

We used a modified version of the QUADAS tool (Whiting 2003), a quality assessment tool for use in systematic reviews of diagnostic accuracy studies, to assess the methodological quality of included studies. We anticipated that a key methodological issue would be the potential for bias arising from the differential use of invasive testing and follow-up for the reference standard according to index test results, bias arising due to higher loss to miscarriage in

false negatives than true negatives. We chose to code this issue as originating from differential verification in the QUADAS tool: we are aware that it could also be coded under delay in obtaining the reference standard, and reporting of withdrawals. We omitted the QUADAS item assessing quality according to length of time between index and reference tests, as Down's syndrome is either present or absent rather than a condition that evolves and resolves, and disregarding the differential reference standard issue thus any length of delay is acceptable. Two review authors assessed each included study separately. Any disagreement between the two authors was settled by consensus, or where necessary, by a third party. Each item in the QUADAS tool was marked as 'yes', 'no' or 'unclear', and scores were summarised graphically. We did not use a summary quality score.

QUADAS criteria included the following 10 questions.

1. Was the spectrum of women representative of the women who will receive the test in practice? (Criteria met if the sample was selected from a wide range of childbearing ages, or selected from a specified 'high risk' group such as over 35s, family history of Down's syndrome, multiple pregnancy or diabetes mellitus, provided all affected and unaffected fetuses included that could be tested at the time point when the screening test would be applied; criteria not met if the sample taken from a select or unrepresentative group of women (i.e. private practice), was an atypical screening population or recruited at a later time point when selection could be affected by selective fetal loss.)
2. Is the reference standard likely to correctly classify the target condition? (Amniocentesis, chorionic villus sampling, postnatal karyotyping, miscarriage with cytogenetic testing of the fetus, a phenotypically normal baby or birth registers are all regarded as meeting this criteria.)
3. Did the whole sample or a random selection of the sample receive verification using a reference standard of diagnosis?
4. Did women receive the same reference standard regardless of the index test result?
5. Was the reference standard independent of the index test result (i.e. the index test did not form part of the reference standard)?
6. Were the index test results interpreted without knowledge of the results of the reference standard?
7. Were the reference standard results interpreted without knowledge of the results of the index test?
8. Were the same clinical data (i.e. maternal age and weight, ethnic origin, gestational age) available when test results were interpreted as would be available when the test is used in practice?
9. Were uninterpretable/intermediate test results reported?
10. Were withdrawals from the study explained?

Statistical analysis and data synthesis

We initially examined each test or test strategy at each of the common risk thresholds used to define test positivity by plotting estimates of sensitivity and specificity from each study on forest plots and in receiver operating characteristic (ROC) space. Test strategies were selected for further investigation if they were evaluated in four or more studies or, if there were three or fewer studies, but the individual study results indicated performance likely to be superior to a sensitivity of 70% and specificity of 90%.

Estimation of average sensitivity and specificity

The analysis for each test strategy was undertaken first, by restricting to studies that reported a common threshold to estimate average sensitivity and specificity for each test at each threshold. Although data on all thresholds were extracted, we present only key common thresholds close to risks of 1:384, 1:250 and the 5% false positive rate (FPR), unless other thresholds were more commonly reported. Where combinations of tests were used in a risk score, we extracted the result for the test combination using the risk score and not the individual components that made up the test.

Meta-analyses were undertaken using hierarchical summary ROC (HSROC) models, which included estimation of random-effects in accuracy and threshold parameters when there were four or more studies. Otherwise, average sensitivity and specificity values were computed by using univariate random-effects logistic regression models to average logit sensitivity and logit specificity separately because of insufficient number of studies to reliably estimate all the parameters in the HSROC model. It is common in this field for studies to report sensitivity for a fixed specificity (usually a 5% FPR). This removes the requirement to account for the correlation between sensitivity and specificity across studies by using a bivariate meta-analytical method since all specificities are the same value. Thus, at a fixed specificity value, logit sensitivities were pooled using a univariate random-effects model. This model was further simplified to a fixed-effect model when there were only two or three studies and heterogeneity was not observed on the SROC plot. All analyses were undertaken using the NLMIXED procedure in SAS (version 9.2; SAS Institute, Cary, NC) and the xtmelogit command in Stata version 11.2 (Stata-Corp, College Station, TX, USA).

Comparisons between tests

Comparisons between tests were first made utilising all available studies, selecting one threshold from each study to estimate a summary ROC curve without restricting to a common threshold. The threshold was chosen for each study according to the following order of preference: a) the risk threshold closest to one in 250; b) a multiples of the median (MoM) or presence/absence threshold; c) the performance closest to a 5% FPR or 95th percentile. The 5% FPR was chosen as a cut-off point as this is the cut-off most commonly reported in the literature. The analysis that used all available studies was performed by including the six most evaluated test strategies in a single HSROC model. The model included

two indicator terms for each test to allow for differences in accuracy and threshold. As there were few studies for each test, a single summary ROC shape parameter was included in the model such that the fitted summary ROC curves did not cross. An estimate of the sensitivity of each test for a 5% FPR was derived from the summary ROC curve, and associated confidence intervals were obtained using the delta method.

Direct comparisons between tests were based on results of very few studies, and were analysed using a fixed-effect HSROC model with symmetrical underlying summary ROC curves because the number of studies was insufficient to estimate between-study heterogeneity in accuracy and threshold or asymmetry in the shape of the summary ROC curves. A separate model was used to make each pair-wise comparison. Comparisons between tests were assessed by using likelihood ratio tests to test if the differences in accuracy were statistically significant or not. The differences were expressed as relative diagnostic odds ratios and were reported with 95% confidence intervals. As studies rarely report data cross-classified by both tests for Down's and normal pregnancies, the analytical method did not take full account of the pairing of test results, but the restriction to direct head-to-head comparisons should have removed the potential confounding of test comparisons with other features of the studies. The strength of evidence for differences in performance of test strategies relied on evidence from both the direct and indirect comparisons.

Investigations of heterogeneity

If there were 10 or more studies available for a test, we planned to investigate heterogeneity by adding covariate terms to the HSROC model to assess the effect of a covariate on accuracy and threshold.

Sensitivity analyses

Mothers with pregnancies identified as high risk for Down's syndrome by ultrasound and serum testing are often offered immediate definitive testing by amniocentesis, whereas those considered low risk are assessed for Down's syndrome by inspection at birth. Such delayed and differential verification will introduce bias, most likely through there being greater loss to miscarriage in the Down's syndrome pregnancies that were not detected by the ultrasound and serum testing (the false negative diagnoses). Testing and detection of miscarriages is impractical in many situations, and no clear data are available on the magnitude of these miscarriage rates. To account for the possible bias introduced by such a mechanism, we planned to perform sensitivity analyses by increasing the percentage of false negatives in studies where delayed verification in test negatives occurred (Mol 1999). We planned to incrementally increase the percentage from 10% to 50%, the final value representing a scenario where a third of more Down's pregnancies than normal pregnancies were likely to miscarry, thought to be higher than the likely value. We intended to conduct the sensitivity analyses on the analysis investigating the effect of maternal age on test sensitivity.

Assessment of reporting bias

Assessment of reporting bias was not performed.

RESULTS

Results of the search

The search for the whole suite of reviews identified a total of 15,394 papers, once the results from each bibliographic database were combined and duplicates were removed. After screening out obviously irrelevant papers based on their title and abstract, 1145 papers remained and we obtained full-text copies for formal assessment of eligibility. From these a total of 269 papers were deemed eligible and were included in the suite of reviews. A total of 22 studies (reported in 25 publications) were included in this review of first and second trimester serum screening, with and without ultrasound, involving 228,615 pregnancies including 1067 Down's syndrome pregnancies.

A total of 32 different test strategies combinations were evaluated in the 22 studies. The tests were produced from combinations of eight different tests, with and without maternal age; first trimester nuchal translucency (NT) and the serum markers AFP, uE3, total hCG, free βhCG, Inhibin A, PAPP-A and ADAM 12. We examined tests combining first and second trimester markers with or without ultrasound as complete tests, and also examined stepwise and contingent strategies. The studies evaluated the following serum-only tests: one single test without maternal age, and one septuple test, two sextuple tests, five quintuple tests, two quadruple tests and two triple test in combination with maternal age. Serum and ultrasound markers were evaluated in combination with maternal age: one study of seven markers, three studies of six markers, four studies of five markers, four studies of four markers and two studies of three markers. In addition, there were two contingent and three stepwise test strategies. Twelve of the 22 studies only evaluated the performance of a single test or test strategy, five compared two tests, two compared three tests, two compared five tests, and one compared 20 tests (Wald 2003b).

The following test combinations were the most evaluated and were each evaluated in four studies.

Six markers

- First trimester NT, first trimester PAPP-A , second trimester free ßhCG, second trimester uE3, second trimester AFP, second trimester Inhibin A, and maternal age (four studies; 40,348 women including 266 Down's syndrome pregnancies)

Four markers

- First trimester PAPP-A, second trimester total hCG, second trimester uE3, second trimester AFP and maternal age (four studies; 2474 women, including 236 Down's syndrome pregnancies)
- First trimester NT, second trimester total hCG, second trimester uE3, second trimester AFP and maternal age (four studies; 13,708 women, including 136 Down's syndrome pregnancies)

Three markers

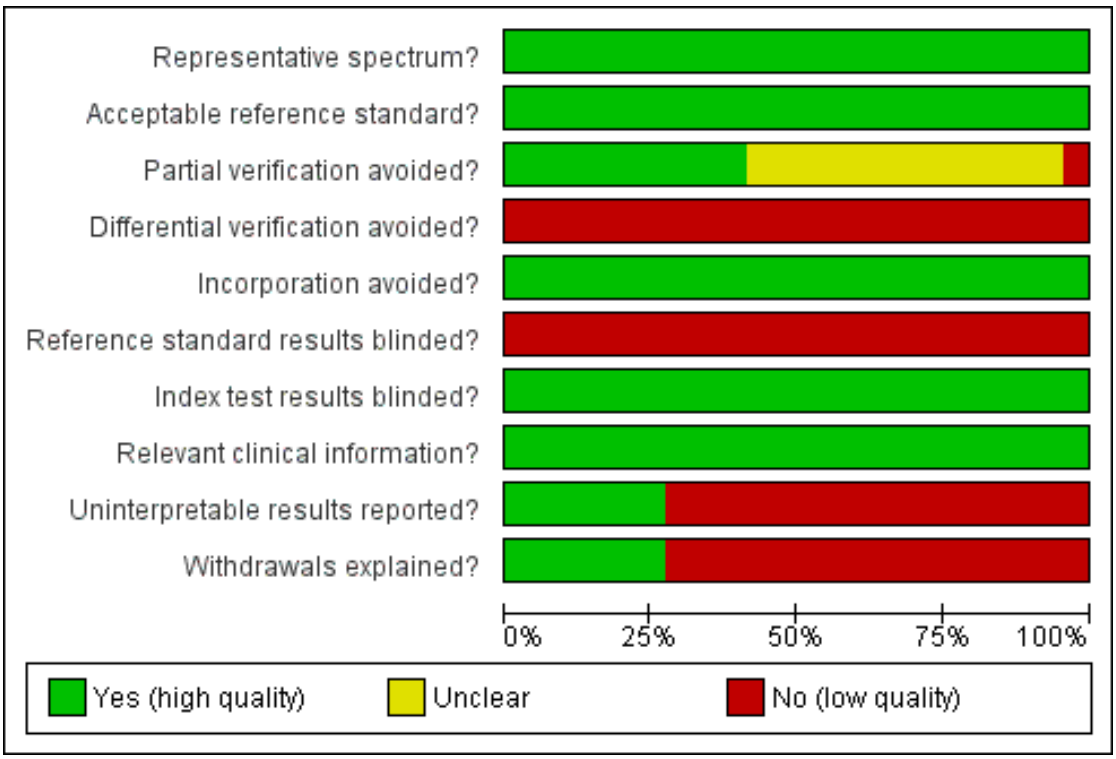
- First trimester NT, second trimester total hCG, second trimester AFP and maternal age (four studies; 22,793 women, including 135 Down's syndrome pregnancies)

Of the remaining 28 test combinations, two were evaluated in three studies, eight were evaluated in two studies and the remaining 18 in single studies only.

Methodological quality of included studies

Methodological quality of the studies was judged to be high in half of the categories (Figure 1). Due to the nature of testing for Down's syndrome screening and the potential side effects of invasive testing, differential verification is almost universal in the general screening population, as most women whose screening test result is defined as low risk will have their screening test verified at birth, rather than by invasive diagnosis in the antenatal period. Additionally, it was not possible to ascertain from the included studies whether or not the results of index tests and reference standards were blinded. It would be difficult to blind clinicians performing invasive diagnostic tests (reference standards) to the index test result, unless all women received the same reference standard, which would not be appropriate in most scenarios. Any biases secondary to a lack of clinician blinding are likely to be minimal.

Figure 1. Methodological quality graph: review authors' judgements about each methodological quality item presented as percentages across all included studies.



Although not explicitly stated, most studies seemed to indicate 100% follow-up. Inevitably there will be losses to follow-up due to, for example, women moving out of the area of a study. It was therefore difficult to measure reporting of uninterpretable tests and hence reporting of withdrawals. Studies usually accounted for these and it is unlikely to have introduced significant bias. There was definitely under-ascertainment of miscarriage, and very few papers accounted for miscarriage or performed tissue karyotyping in pregnancies resulting in miscarriage. Some studies attempted to adjust for predicted miscarriage rate and the incidence of Down's syndrome in this specific population, but most did not. We have not attempted to adjust for expected miscarriage rate in this review. This issue has the potential to have more influence with first trimester testing due to a higher miscarriage rate per se in this trimester.

Some studies that provided estimates of risk using multivariable equations used the same data set to evaluate performance of the risk equation as was used to derive the equation. This is often thought to lead to over-estimation of test performance.

Findings

The results for the six most evaluated test strategies are presented in Summary of findings 1. Additional information is provided below.

1) First trimester nuchal translucency, first trimester PAPP-A, second trimester free ßhCG, second trimester uE3, second trimester AFP, second trimester Inhibin A, and maternal age

Four studies (Aagaard-Tillery 2009; Bestwick 2010; Wald 2003b; Wald 2009) evaluated this test strategy. The studies included 40,348 women in whom 266 pregnancies were affected by Down's syndrome. Over half the data were provided by Bestwick 2010 (22,746 women, including 106 Down's syndrome pregnancies). Studies presented data for different cut-points but three (Aagaard-Tillery 2009; Bestwick 2010; Wald 2003b) of the four studies also presented data for a 5% false positive rate (FPR). At a fixed cut-point of 5% FPR on the summary ROC curve, the estimated sensitivity based on all four studies was 92% (95% confidence interval (CI) 88 to 95).

2) First trimester PAPP-A, second trimester total hCG, second trimester uE3, second trimester AFP and maternal age

Four studies (Baviera 2010; Wald 2003b; Wright 2010 FASTER trial; Wright 2010 North York) evaluated this test strategy. The studies included 2474 women in whom 236 pregnancies were affected by Down's syndrome. Most of the data were provided by Wald 2003b (118 women, including 98 Down's syndrome pregnancies). Studies presented data for cut-points of 5% FPR (two studies Baviera 2010; Wald 2003b) and 1:250 risk (two studies Wright 2010 FASTER trial; Wright 2010 North York). At a fixed cut-point of 5% FPR, the estimated sensitivity was 85% (95% CI 78 to 89).

3) First trimester nuchal translucency, second trimester total hCG, second trimester uE3, second trimester AFP and maternal age

Results for this test strategy were derived from four studies (Babbur 2005; Herman 2002; Schuchter 2001; Wald 2003b) and included 13,708 women in whom 136 pregnancies were known to be affected by Down's syndrome. Schuchter 2001 contributed 9342 pregnancies to the data. Studies presented data for cut-points of 5% FPR (two studies: Schuchter 2001; Wald 2003b) and 1:250 risk (two studies:Babbur 2005; Herman 2002). At a fixed cut-point of 5% FPR, the estimated sensitivity was 86% (95% CI 78 to 92).

4) First trimester nuchal translucency, second trimester total hCG, second trimester AFP and maternal age

Results were derived from four studies (Audibert 2001; Benattar 1999; Lam 2002; Wald 2003b) and included 22,793 women in whom 135 pregnancies were known to be affected by Down's syndrome. Lam 2002 contributed 16,237 pregnancies to the data. Studies presented data for cut-points of 5% FPR (two studies: Lam 2002; Wald 2003b;) and 1:250 risk (two studies: Audibert 2001; Benattar 1999). At a fixed cut-point of 5% FPR, the estimated sensitivity was 85% (CI 77 to 91).

5) Other test combinations

Of the 28 test combinations evaluated in three or fewer studies, 25 test combinations demonstrated estimated sensitivities of at least 70% and estimated specificities of more than 90%. Sixteen of these were evaluated in single studies only (see Summary of findings 2). Of the remaining nine test combinations evaluated in two or three studies, data were pooled for the following six tests.

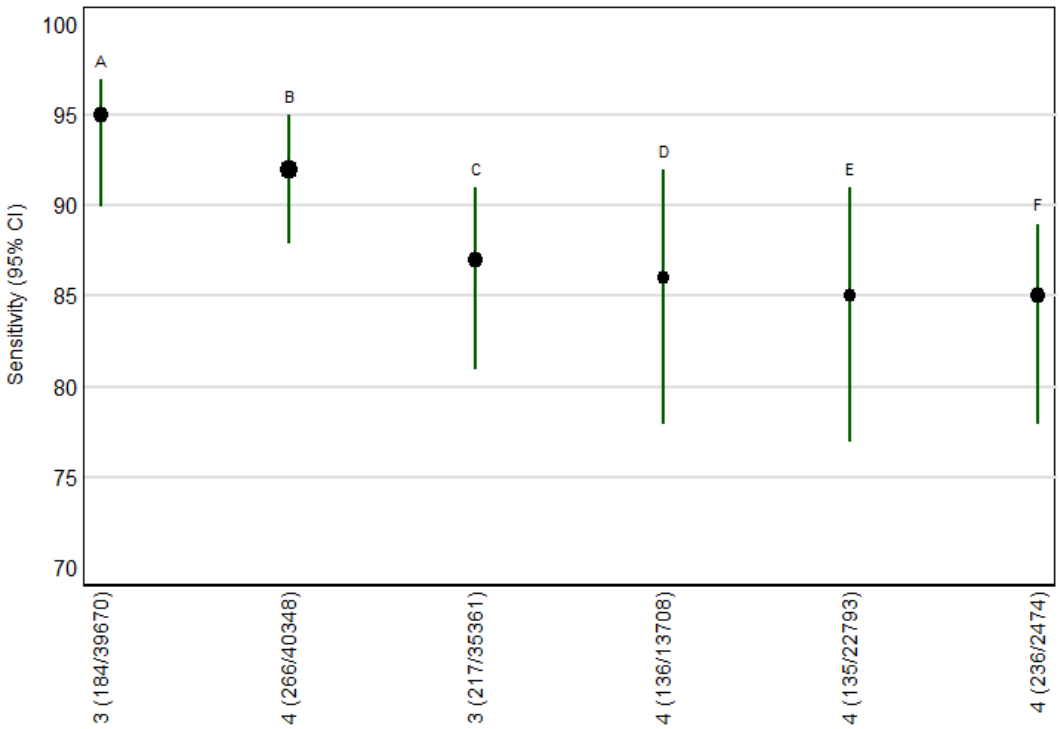
- **First trimester PAPP-A and second trimester total hCG, uE3, AFP and PAPP-A, and maternal age** evaluated in two studies (Wright 2010 FASTER trial; Wright 2010 North York) estimated a sensitivity of 78% (CI 66 to 86) and specificity of 98% (CI 96 to 99) at a cut-point of 1:200 risk.
- **First trimester PAPP-A and second trimester total hCG, uE3, AFP and inhibin A, and maternal age** evaluated in three studies (Malone 2005; Palomaki 2006; Wald 2003b) estimated a sensitivity of 87% (CI 81 to 91) at a cut-point of 5% FPR.
- **First trimester PAPP-A and total hCG, and second trimester total hCG, uE3 and AFP** evaluated in two studies (Wright 2010 FASTER trial; Wright 2010 North York) estimated a sensitivity of 80% (CI 68 to 88) and specificity of 97% (CI 94 to 98) at a cut-point of 1:200 risk.

- **First trimester PAPP-A and uE3, and second trimester total hCG, uE3 and AFP** evaluated in two studies (Wright 2010 FASTER trial; Wright 2010 North York) estimated a sensitivity of 80% (CI 68 to 88) and specificity of 96% (CI 93 to 98) at a cut-point of 1:200 risk.
- **First trimester NT and second trimester free ßhCG and AFP, and maternal age** evaluated in two studies (Rozenberg 2002; Wald 2003b) estimated a sensitivity of 83% (CI 70 to 91) at a cut-point of 5% FPR.
- **First trimester NT and PAPP-A, and second trimester total hCG, uE3, AFP and Inhibin A, and maternal age** evaluated in three studies (Malone 2005; Wald 2003b; Wald 2009) estimated a sensitivity of 95% (CI 90 to 97) at a cut-point of 5% FPR.

Comparative analysis of the six selected test strategies

For each test, we obtained the detection rate (sensitivity) for a fixed false positive rate (FPR) (1-specificity), a metric which is commonly used in Down's syndrome screening to describe test performance. We chose to estimate detection rates at a 5% FPR in common with much of the literature. Figure 2 shows point estimates of the detection rate (and their 95% CIs) at a 5% FPR based on all available data for the six test strategies; the test strategies are ordered according to decreasing detection rates. The plot shows that all six test strategies have detection rates of 85% and above. The six marker maternal age-adjusted combination of first trimester NT and PAPP-A with second trimester total hCG, uE3, AFP and inhibin A showed the highest detection rate with an estimated detection rate of 95% (95% CI 90 to 97) based on data from three studies with 184 affected cases out of a total of 39,670 pregnancies. The next best performing strategy was a test combination with the same markers except that it included free ßhCG instead of total hCG. For this combination, the estimated detection rate was 92% (95% CI 88 to 95) based on data from four studies with 266 affected cases out of a total of 40,348 pregnancies. The remaining four test strategies showed similar detection rates.

Figure 2. Detection rates (% sensitivity) at a 5% false positive rate for the six most evaluated test strategies (estimates from summary ROC curves).A = First trimester NT and PAPP-A , second trimester total hCG, uE3, AFP and inhibin A;B = First trimester NT and PAPP-A , second trimester free ßhCG, uE3, AFP and inhibin A; C = First trimester PAPP-A , second trimester total hCG, uE3, AFP and inhibin A; D = First trimester NT, second trimester total hCG, uE3 and AFP; E = First trimester NT, second trimester total hCG and AFP; and F = First trimester PAPP-A , second trimester total hCG, uE3 and AFP.All test combinations include maternal age. Each circle represents the summary sensitivity for a test strategy at a 5% false positive rate. The size of each circle is proportional to the number of Down's cases. The estimates are shown with 95% confidence intervals. The test strategies are ordered on the plot according to decreasing detection rate. The number of studies, cases and women included for each test strategy are shown on the horizontal axis.



The strength of evidence for differences in the diagnostic performance of the six test strategies relied on evidence from both direct and indirect comparisons. Table 1 shows pair-wise direct comparisons (head-to-head) where studies were available. Such comparisons are regarded as providing the strongest evidence as differences between tests are unconfounded by study characteristics. The table shows the number of studies (*K*), the ratios of diagnostic odds ratios (DORs) with 95% CI and P values for each test comparison. There were no statistically significant differences in accuracy between any pair of tests. However, all comparisons in this table were based on one or two studies and so are unlikely to be powered to detect differences in accuracy.

Table 2 shows the same comparisons made using all available data. Results are generally in agreement with the direct comparisons, and in addition, showed some statistically significance differences ($P < 0.05$) suggesting that the six marker maternal age-adjusted combination of first trimester NT and PAPP-A with second trimester total hCG, uE3, AFP and inhibin A outperformed all the other test strategies except when compared with a similar strategy that included free ßhCG instead total hCG.

Comparison of integrated, contingent and stepwise strategy for a septuple combination of serum tests and first trimester nuchal translucency

Table 3 shows the results of two studies that assessed integrated, contingent or stepwise strategies. Integrated testing involves performing first trimester NT, PAPP-A and free ßhCG, and second trimester uE3, AFP, total hCG and inhibin A, without disclosure of the first trimester result. The strategy was evaluated in one study (Malone 2005) that reported a 94% sensitivity (95% CI 87 to 98) and 89% specificity (95% CI 89 to 89) for a cut-point of 1:150.
In one study (Cuckle 2008), stepwise and contingent tests were compared in the same patient population, with similar detection rates (stepwise 91% (95% CI 84 to 97); contingent 92% (95% CI 82 to 96)) and identical false positive rates of 5% at cut-points of 1:270.
The perceived advantages of the stepwise and contingent methods are that women deemed to be very high risk are offered invasive testing in the first trimester, allowing for earlier detection of Down's syndrome and subsequent management. Termination of pregnancy in the first trimester of pregnancy is safer than at later gestations. With contingent screening, where women are deemed to be low risk with a numerical risk of < 1:1500, no further testing is offered, and it does not appear to adversely affect the detection rate. In those women who are considered to be intermediate risk, additional second trimester serum tests may detect cases of Down's syndrome that would have been missed. Of note, in the study evaluated, all of the women found to have a risk of > 1:30 on first trimester screening were found to be high risk upon completion of the full contingent screening package. This type of screening may facilitate patient decision making, however further evaluation needs to be carried out.
It is difficult to make a comparison between the integrated method and the stepwise and contingent methods in practical terms, as the non-disclosure of the first trimester result means that women would not be offered earlier diagnostic testing. More information is required about all three methods of testing in order to make a recommendation, as not all methods will be acceptable to women.

Investigation of heterogeneity and sensitivity analyses

The key characteristics of the 22 included studies is summarised in Table 4 with further details available in the Characteristics of included studies table. None of the tests was evaluated by 10 or more studies and so we were unable to investigate the effect of any potential source of heterogeneity. The planned sensitivity analyses were also not possible.

Summary of findings

Test strategy (with maternal age)	Studies	Women (cases)	Sensitivity (95% CI) at a 5% FPR	Test*
First trimester PAPP-A and second trimester total hCG, uE3 and AFP	4	2474 (236)	85 (78, 89)	P = 0.014
First trimester PAPP-A and second trimester total hCG, uE3, AFP and inhibin A	3	35,361 (217)	87 (81, 91)	
First trimester NT and second trimester total hCG and AFP	4	22,793 (135)	85 (77, 91)	
First trimester NT and second trimester total hCG, uE3 and AFP	4	13,708 (136)	86 (78, 92)	
First trimester NT and PAPP-A, and second trimester total hCG, uE3, AFP and inhibin A	3	39,670 (184)	95 (90, 97)	
First trimester NT and PAPP-A, and second trimester free ßhCG, uE3, AFP and inhibin A	4	40,348 (266)	92 (88, 95)	

*Likelihood ratio test for the difference in accuracy between the six test strategies compared in a single meta-analytic model
AFP = alpha-fetoprotein; **ßhCG** = beta human chorionic gonadotrophin; **FPR** = false positive rate;**hCG** = human chorionic gonadotrophin; **NT** = nuchal translucency; **PAPP-A** = pregnancy-associated plasma protein-A; **uE3** = unconjugated oestriol
CI = confidence interval

Test	Studies	Women (cases)	Sensitivity* (95% CI)	Specificity* (95% CI)	Threshold
Without maternal age and ultrasound					
Single tests					
ADAM 12 second trimester to first trimester ratio	1	579 (17)	53 (28, 77)	95 (93, 97)	5% FPR
With maternal age and without ultrasound					
Triple tests					
First trimester PAPP-A and second trimester total hCG and AFP	1	1188 (98)	83 (74, 90)	95 (93, 96)	5% FPR
First trimester PAPP-A and second trimester free ßhCG and AFP	2	2197 (94)	83 to 85	94 to 95	5% FPR, 1:300 risk
Quadruple tests					
First trimester PAPP-A and second trimester free ßhCG, uE3 and AFP	1	1188 (98)	86 (77, 92)	95 (93, 96)	5% FPR
Quintuple tests					
First trimester PAPP-A and second trimester free ßhCG, uE3, AFP and inhibin A	1	1188 (98)	90 (82, 95)	95 (93, 96)	5% FPR

First trimester PAPP-A and second trimester total hCG, uE3, AFP and PAPP-A	2	707 (121)	78 (66, 86)	98 (96, 99)	1:200 risk
First trimester PAPP-A and total hCG, and second trimester total hCG, uE3 and AFP	2	707 (121)	80 (68, 88)	97 (94, 98)	1:200 risk
First trimester PAPP-A and uE3, and second trimester total hCG, uE3 and AFP	2	707 (121)	80 (68, 88)	96 (93, 98)	1:200 risk
Sextuple tests					
First trimester AFP, free ßhCG and uE3, and second trimester total hCG, uE3 and AFP	1	12,339 (34)	82 (65, 93)	94 (93, 94)	1:250 risk
First trimester PAPP-A and second trimester total hCG, uE3, AFP, inhibin A and PAPP-A	1	540 (32)	84 (67, 95)	96 (94, 98)	1:250 risk
Septuple tests					
First trimester PAPP-A, total hCG and uE3, and second trimester total hCG, uE3, AFP and PAPP-A	2	707 (121)	49 (36, 61)	98 (96, 99)	1:200 risk
With maternal age and ultrasound					
Triple tests					

First trimester NT and second trimester free ßhCG and AFP	2	6616 (105)	83 (70, 91)	95	5% FPR
Quadruple tests					
First trimester NT and second trimester free ßhCG, uE3 and AFP	1	1110 (85)	88 (79, 94)	95 (94, 96)	5% FPR
First trimester NT and PAPP-A, and second trimester total hCG and AFP	1	1110 (85)	91 (82, 96)	95 (94, 96)	5% FPR
First trimester NT and PAPP-A, and second trimester free ßhCG and AFP	2	3400 (93)	88 to 91	95 to 98	5% FPR, 1:300 risk
Quintuple tests					
First trimester NT and second trimester total hCG, uE3, AFP and inhibin A	1	1110 (85)	91 (82, 96)	95 (94, 96)	5% FPR
First trimester NT and second trimester free ßhCG, uE3, AFP and inhibin A	1	1110 (85)	91 (82, 96)	95 (94, 96)	5% FPR
First trimester NT and PAPP-A, and second trimester free ßhCG, uE3 and AFP	1	1100 (85)	92 (84, 97)	95 (94, 96)	5% FPR

First trimester NT and PAPP-A, and second trimester total hCG, uE3 and AFP	2	33,337 (171)	88 to 92	95 to 97	5% FPR, 1:200 risk
Sextuple tests					
First trimester NT, PAPP-A and free ßhCG, and second trimester total hCG, uE3 and AFP	1	5060 (13)	100 (75, 100)	97 (96, 97)	1:250 risk
Septuple tests					
First trimester NT, PAPP-A and free ßhCG, and second trimester uE3, AFP, total hCG and inhibin A	1	33,546 (87)	94 (87, 98)	89 (89, 89)	1:150 risk
Contingent tests					
First trimester NT, PAPP-A and free ßhCG, if risk 1:30-1:1500, second trimester total hCG, uE3, AFP and inhibin A	1	32,355 (86)	91 (82, 96)	95 (95, 96)	1:270 risk
First trimester NT, PAPP-A and free ßhCG, if risk 1:30-1:1500, second trimester free ßhCG, uE3, AFP and inhibin A	1	7842 (59)	95 (86, 99)	95 (94, 95)	5% FPR
Stepwise tests					

First trimester NT and PAPP-A, if risk < 1:100, second trimester free ßhCG, uE3 and AFP	1	1507 (12)	92 (62, 100)	97 [(96, 98)	1:250 risk
First trimester NT, PAPP-A and free ßhCG, if risk < 1:30, second trimester total hCG, uE3, AFP and inhibin A	1	32,355 (86)	92 (84, 97)	95 (95, 95)	1:270 risk
First trimester NT, PAPP-A and free ßhCG, if risk < 1:30, second trimester free ßhCG, uE3, AFP and 2T inhibin A	1	7842 (59)	97 (88, 100)	95 (94, 95)	5% FPR

*Tests evaluated by at least one study are presented in the table. Where there were two studies at the same threshold, estimates of summary sensitivity and summary specificity were obtained by using univariate fixed-effect logistic regression models to pool sensitivities and specificities separately. if the threshold used was a 5% FPR, then only the sensitivities were pooled. The range of sensitivities and specificities are presented where there were two studies and the thresholds used were different.

AFP = alpha-fetoprotein; **ßhCG** = beta human chorionic gonadotrophin; **FPR** = false positive rate; **hCG** = human chorionic gonadotrophin; **NT** = nuchal translucency; **PAPP-A** = pregnancy-associated plasma protein-A; **uE3** = unconjugated oestriol
CI = confidence interval

DISCUSSION

Summary of main results

We found 22 studies evaluating first and second trimester Down's syndrome serum screening tests, with or without first trimester nuchal translucency (NT). Few studies provided unconfounded comparisons of test strategies by applying and comparing several strategies using the same serum sample, the majority of studies only evaluating a single test combination. A summary of results for the six most commonly evaluated test strategies is given in Summary of findings 1, and the remaining 26 test strategies are given in Summary of findings 2.

Three key findings were noted.

1. The combined test comprised of first trimester NT and PAPP-A, and second trimester total hCG, uE3, AFP and Inhibin A, and maternal age evaluated in three studies (Malone 2005; Wald 2003b; Wald 2009) estimated a sensitivity of 95% (confidence interval (CI) 90 to 97) at a cut-point of 5% FPR. In indirect comparisons this test combination significantly outperformed all others, except the test combination of first trimester NT, first trimester PAPP-A, second trimester free ßhCG, second trimester uE3, second trimester AFP, second trimester Inhibin A, and maternal age with a sensitivity of 92% (95% CI 88 to 95) for a fixed 5% FPR.
2. In direct comparisons of tests in the same population of women, no test was found to be significantly better. These comparisons were based on one or two studies, and are therefore unlikely to be powered to detect differences.
3. Stepwise and contingent screening strategies show promising detection rates for fixed FPRs, however due to the nature of the test strategies it is not appropriate to make comparisons between these tests and those that do not involved stratification or risk at several different points in the screening journey. These test strategies warrant further study.

Strengths and weaknesses of the review

This review is the first comprehensive review of first and second trimester serum and ultrasound screening. We examined papers from around the world, covering a wide cross-section of women in varying populations. We contacted authors to verify data where necessary to give as complete a picture as possible while trying to avoid replication of data.

There were a number of factors that made meta-analysis of the data difficult, which we tried to adapt for in order to allow for comparability of data presented in different studies.

1. There were many different cut-points used to define pregnancies as high or low risk for Down's syndrome. This means that direct comparison is more difficult than if all studies used the same cut-point to dichotomise their populations.
2. There were many different risk equations and software applications in use for combination of multiple markers, which were often not described in the papers. This means that risks may be calculated by different formulae and they may not be directly comparable for this reason. It is possible that this is responsible for unexplained heterogeneity in results.
3. Different laboratories and clinics run different assays and use different machines and methods. This may influence raw results and subsequent risk calculations. Many laboratories have a quality assessment or audit trail, however, this may not necessarily be standard across the board. For example, how many assays are run, how often medians are calculated and adjusted for a given population and how quickly samples are tested from initially being taken.
4. Few studies made direct comparisons between tests, making it difficult to detect if a real difference exists between tests (i.e. how different tests perform in the same population). There were differences in populations, with assay medians being affected, for example, by race. It is not certain whether it is appropriate to make comparisons between populations which are inherently different.
5. We were unable to perform the investigations of heterogeneity that we had originally intended to because the data simply were not available. The vast majority of papers looking at pregnancies conceived by IVF, affected by diabetes, multiple gestation or a family history of Down's syndrome involved unaffected pregnancies only.

In addition, the search for this review was last updated in August 2011, and it is possible that new studies may have been published which have not been included. Since the search was completed we have kept a watching brief on outputs and are not aware of any studies with large sample sizes which could substantially affect the findings.

Applicability of findings to the review question

Potentially, when planning screening policy or a clinical screening programme, clinicians and policy makers need to make decisions about a finite number of tests or type of tests that can be offered. These policies are often driven by both the needs of a specific population and by financial resources. Economic analysis was considered to be outside of the scope of this review. Many of the tests examined as part of this review are already commercially available and in use in the clinical setting. The studies were carried out on populations of typical pregnant women and therefore, the results should be considered comparable with most pregnant populations encountered in every day clinical practice.

We were unable to extract information about harms of testing, information about miscarriage rates and uptake of definitive testing as the data were not available the majority of the time. While it is unlikely that major differences between the tests evaluated here exist in terms of direct harms of testing, as they are all based on

ultrasound, with or without a blood sample, differences in accuracy may lead to differences in the use of definitive testing and its consequent adverse outcomes.

In some countries with a defined screening policy (i.e. the UK), first trimester screening plays a major role, usually in combination with first trimester ultrasound scanning, and second trimester serum screening is also readily available. In other countries however, there may only be a limited range of tests or markers available-often second trimester markers, rather than first trimester markers. The results of this review should be interpreted and applied in the context of test availability and local restrictions, populations or policies.

AUTHORS' CONCLUSIONS

Implications for practice

The evidence supports the use of the six marker maternal age-adjusted combination of first trimester nuchal translucency (NT) and pregnancy-associated plasma protein-A (PAPP-A) with second trimester total human chorionic gonadotrophin (hCG), unconjugated oestriol (uE3), alpha-fetoprotein (AFP) and inhibin A, which outperformed other test strategies. However the evidence was limited, based on small numbers of studies and the finding was not demonstrated in direct comparison of markers in the same populations of women. We cannot recommend a single test combination for Down's syndrome screening. The choice of multiple markers will depend on the availability of certain assays in local laboratories. There is little evidence to recommend the use of first and second trimester serum markers without the addition of first trimester ultrasound. We would not recommend that these tests be introduced into wider clinical practice without careful consideration of cost.

Implications for research

Further evaluation of test combinations involving contingent and stepwise strategies are required to determine whether they offer superior test performance.

Future studies should ensure that adequate sample sizes are recruited, and take opportunities to make comparisons of test performance testing several alternative test combinations on the same population. Such direct comparison removes issues of confounding when making test comparisons, and allows a clear focus on testing the incremental benefit of increasingly complex and expensive testing strategies. The reporting of studies of test accuracy can be improved and more closely adhere to the standards for the reporting of diagnostic accuracy studies (STARD) guideline. Three key aspects of this are: 1) formally testing the statistical significance of differences in test performance in direct comparisons and estimating incremental changes in detection rates (together with confidence intervals); 2) clearly reporting the number of mothers studied and their results; and 3) reporting the numbers of women who are lost to follow-up. Many authors reported results of extrapolating findings to age-standardised national cohorts to demonstrate the performance of the test, and failed to report the actual numbers studied and evaluated.

For the purposes of meta-analysis and to allow for comparisons to be made between different tests and combinations, we would recommend the publication of consensus standard algorithms for estimating risk, and reporting of test performance at a standard set of thresholds. This would be difficult to achieve and implement, but an attempt at consensus should be made.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Aagaard-Tillery 2009

Clinical features and settings	Routine screening
Participants	7842 participants who underwent both first and second trimester screening and a second trimester genetic sonogram USA - The First and Second Trimester Evaluation of Risk (FASTER) trial (13 centres) Dates not specified Pregnant women Mean maternal age 30.6 years (SD 6.1 years) Singleton pregnancies 11-13 and 15-23 weeks' gestation
Study design	Prospective cohort study
Target condition and reference standard(s)	Down's syndrome: 59 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT, PAPP-A and free ßhCG (details not reported) Second trimester AFP, uE3, free ßhCG and inhibin-A (details not reported) Second trimester genetic sonogram Detection rate for a 5% false positive rate and for fixed 1:270 cut-off
Follow-up	Details of follow-up not reported
Aim of study	To estimate the effectiveness of second trimester genetic sonography in modifying Down's syndrome screening results
Test characteristics	
Reference standard used	
Notes	

Table of Methodological Quality *Table of Metho*

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth

Aagaard-Tillery 2009 (*Continued*)

Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	Yes	Of 33,546 trial participants only 7842 women with complete information for all screening tests and genetic sonography were included in the study

Audibert 2001

Clinical features and settings	Routine screening
Participants	4130 participants France - single centre May 1994 to December 1997 Pregnant women Mean maternal age 30.1 years (all under 38 years) Singleton pregnancies 12-13 weeks' gestation Crown rump length between 38 mm and 84 mm
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome: 12 cases Reference standards: prenatal karyotype conducted (in 7.6% of patients) depending on presence of risk > 1/125, high maternal age, parental anxiety, history of chromosomal defects or parental translocation or abnormal second trimester scan Cytogenetic testing of newborns with suspected abnormalities Postmortum on terminations of pregnancy or miscarriages Follow-up to neonatal examination in newborns

Audibert 2001 (*Continued*)

Index and comparator tests	Maternal age First trimester NT planned at 12-13 weeks, 3 mm cut-off Second trimester serum hCG between 14 and 17 weeks (Amerlite, Orthoclinical diagnostics machine) Second trimester serum AFP between 14 and 17 weeks (Amerlite, Orthoclinical diagnostics machine) Serum tests in 3790 women Risk cut off 1:250
Follow-up	Outcome assessed at delivery and postnatal paediatric examination. 35 women were lost to follow-up and excluded from the analysis. 340 women had first trimester NT but not second trimester serum testing
Aim of study	To compare first trimester NT and second trimester maternal serum measurements as alternative methods of antenatal screening in a low risk population and to evaluate the consequence of combining the results in the estimation of risk
Test characteristics	
Reference standard used	
Notes	Women lost to follow-up were excluded in the final analysis. All detected cases resulted in termination

Table of Methodological Quality ***Table of Metho***

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Yes	All women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results

Audibert 2001 (*Continued*)

Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	Yes	NT was not measured or not recorded in 219 women and these patients were excluded from the study
Withdrawals explained? All tests	Yes	35 women were lost to follow-up (they had all had normal NT results). 340 women who did not want second trimester serum screening withdrew from that part of the study

Babbur 2005

Clinical features and settings	Women requesting screening (self-paying service) and women attending on account of previous pregnancy history of fetal abnormality
Participants	3,188 participants UK - Maternity Hospital August 2001 - March 2004 Pregnant women Singleton pregnancies Median maternal age 37 years (range 19-46 years) 11-14 weeks' gestation 45 mm to 84 mm crown rump length Viable fetus
Study design	Prospective cohort study
Target condition and reference standard(s)	Down's syndrome: 25 cases Reference standards: Invasive testing offered to women with NT > 3 mm or risk > 1: 250 as defined by combined NT and serum results (CVS from 11 weeks, amniocentesis from 15 weeks). Rapid in situ hybridisation test in patients with risk > 1:30. No details given of any follow-up to birth
Index and comparator tests	First trimester NT in all women (FMF methods) Second trimester serum uE3, AFP and hCG (AutoDELFIA(TM) time-resolved fluoroimmunoassay (Perkin Elmer)) at 14 weeks. Offered to patients with negative first trimester NT (n = 2725, 85% accepted)
Follow-up	Details of follow-up not reported
Aim of study	To determine the detection and false positive rates for trisomy 21 using 2-stage combined NT and triple testing whilst disclosing abnormal NT measurements at the scan
Test characteristics	
Reference standard used	
Notes	Women with miscarriages excluded

Babbur 2005 (*Continued*)

Table of Methodological Quality		*Table of Metho*
Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Selective testing of high risk women as done in practice
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	Yes	463 patients having NT did not go on to have second trimester serum testing

Baviera 2010

Clinical features and settings	Routine screening
Participants	579 participants: 17 cases and 562 controls matched for gestational age Italy - single centre December 2006 - May 2009 Pregnant women Mean maternal age 35.3 years (cases) and 30.4 years (controls) Singleton pregnancies 7-10 and 14-17 weeks' gestation
Study design	Case-control study

Baviera 2010 (*Continued*)

Target condition and reference standard(s)	Down's syndrome: 17 cases (14 identified by amniocentesis, 3 from follow-up to birth) Reference standard: amniocentesis or follow-up to birth
Index and comparator tests	Frozen serum samples tested for: First trimester and second trimester ADAM12s (time resolved fluorescence immunoassay, DELFIA assay kit, Perkin Elmer Life and Analytical Sciences) First trimester PAPP-A (details not reported) Second trimester AFP, uE3 and hCG (details not reported)
Follow-up	Details of follow-up not reported
Aim of study	To demonstrate the potential value of repeated measures of ADAM12s for the screening of Down's syndrome
Test characteristics	
Reference standard used	
Notes	

Table of Methodological Quality ***Table of Metho***

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Yes	All women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice

Baviera 2010 (*Continued*)

Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given

Benattar 1999

Clinical features and settings	Routine screening
Participants	1656 participants France - single centre January to December 1995 Pregnant women Singleton pregnancies Mean maternal age 32 years (range 16-46 years) Enrolled before 13 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 5 cases Reference standards: amniocentesis due to maternal age > 38 years (6.1% or women) . Karyotyping encouraged for women with positive result on 1 or more index test. No details of reference standard for index test negative women
Index and comparator tests	Maternal age NT at 12-14 weeks (Toshiba SSA 270), cut-point 1:250 First trimester (12-14 weeks) serum AFP and free ßhCG (Elsa AFP and Elsa free BhCG; Cis-Bio International) Second trimester (15-18 weeks) serum AFP and total hCG (AFP-2T and hCG-60; Ortho-Clinical Diagnostics) All women had NT and serum testing
Follow-up	Details of follow-up not reported. 12 patients were lost to follow-up due to miscarriages
Aim of study	To evaluate the sequential combination of ultrasound screening for fetal aneuploidy at 11-14 weeks with maternal biochemistry at 12-14 and 15-18 weeks of gestation
Test characteristics	
Reference standard used	
Notes	

Table of Methodological Quality ***Table of Metho***

Item	**Authors' judgement**	**Description**

Benattar 1999 (*Continued*)

Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given

Bestwick 2010

Clinical features and settings	Routine screening
Participants	22,746 participants UK - 2 clinics January 2003 - December 2008 Pregnant women Median maternal age 39 years (Down's syndrome) and 34 years (non-Down's syndrome) 11-13 and 14-22 weeks' gestation
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 106 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT, PAPP-A and free ßhCG (details not reported) Second trimester AFP, uE3, free ßhCG and inhibin-A (details not reported)

Bestwick 2010 (*Continued*)

Follow-up	Data obtained from the Hospitals, the regional cytogenetic unit and the National Down Syndrome Cytogenetic Register
Aim of study	To determine whether the SD of NT measurements has decreased over time and, if so, to revise the estimate and assess the effect of revising the estimate of the SD on the performance of antenatal screening for Down's syndrome
Test characteristics	
Reference standard used	
Notes	

Table of Methodological Quality ***Table of Metho***

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given

Cuckle 2008

Clinical features and settings	Routine screening
Participants	36,740 participants undergoing first trimester screening (32,355 also underwent second trimester screening) USA - 15 centres, FASTER trial Pregnant women Singleton pregnancies Maternal age not reported 11-13 and 15-18 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 116 cases (86 cases had both first trimester and second trimester screening) Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT, PAPP-A and free ßhCG (details not reported) Second trimester AFP, total hCG, uE3 and inhibin-A (details not reported)
Follow-up	Details of follow-up not reported
Aim of study	To compare the contingent, step-wise and integrated screening policies
Test characteristics	
Reference standard used	
Notes	

Table of Methodological Quality ***Table of Metho***

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test

Cuckle 2008 (*Continued*)

Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given

Goh 1996

Clinical features and settings	Routine screening
Participants	11,964 participants Singapore - University Hospital 1989 to 1992 Pregnant women Singleton pregnancies Median maternal age 35 years (mean 33 years) 12-22 weeks' gestation
Study design	Cohort
Target condition and reference standard(s)	Down's syndrome: 34 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester and second trimester AFP and hCG (EIA method, kits from Abbot Laboratory, USA) and uE3 (In-house indirect, extraction radioimmunoassay) Risk cut-points of 1:250 and 1:384
Follow-up	No details of methods of follow-up
Aim of study	To appraise the potential effectiveness of implementing a prenatal screening programme on a local population in Singapore
Test characteristics	
Reference standard used	
Notes	

Goh 1996 (*Continued*)

Table of Methodological Quality		*Table of Metho*
Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given

Guanciali-Franchi 2010

Clinical features and settings	Routine screening
Participants	5060 participants Italy - Genetic unit January 2006 - April 2009 Pregnant women Mean maternal age 31.8 years Singleton pregnancies 10-12 and 15-17 weeks' gestation
Study design	Prospective cohort

Guanciali-Franchi 2010 (*Continued*)

Target condition and reference standard(s)	Down's syndrome: 13 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (by certified sonographers) First trimester PAPP-A and free ßhCG (details not reported) Second trimester AFP, hCG and uE3 (details not reported) Cross-trimester test: all first trimester and second trimester tests Cut-point 1:250
Follow-up	Stated that follow-up until delivery was available for all women
Aim of study	To evaluate the effectiveness of cross-trimester testing in selecting high risk pregnant women to undergo invasive prenatal diagnosis
Test characteristics	
Reference standard used	
Notes	

Table of Methodological Quality ***Table of Metho***

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Yes	All women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice

Guanciali-Franchi 2010 (*Continued*)

Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given

Habayeb 2010

Clinical features and settings	Routine screening
Participants	1507 participants UK - Fetal medicine unit September 2007 - December 2008 Pregnant women Median maternal age 35.4 years (range 18-49 years) 9-10, 11-13 and > 14 weeks' gestation
Study design	Cohort
Target condition and reference standard(s)	Down's syndrome: 12 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age Early first trimester PAPP-A (9 weeks' gestation) (AutoDELFIA PAPP-A kit, PerkinElmer LAS (UK) Ltd) First trimester NT (11-13 weeks' gestation) (General Electric E8, Voluson 730 Pro, GE Healthcare) Second trimester AFP, free ßhCG and uE3 (at or after 14 weeks' gestation) (AutoDELFIA (TM) time-resolved fluoroimmunoassay, PerkinElmer Life Sciences) Second trimester tests given if first trimester risk low (< 1:100) or invasive testing declined Cut-point for second-stage risk 1:250
Follow-up	Data recorded on a fetal medicine database and combined with data held on separate databases for pregnancy outcome and the regional cytogenetic laboratory. Cytogenetic test results available for all women delivering in the region
Aim of study	To audit a model combining early PAPP-A with NT and early triple test
Test characteristics	
Reference standard used	
Notes	

Table of Methodological Quality ***Table of Metho***

Item	**Authors' judgement**	**Description**

Habayeb 2010 (*Continued*)

Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given

Herman 2002

Clinical features and settings	Routine screening
Participants	531 participants: 23 cases and 508 consecutive controls Israel - Medical centre Pregnant women 10-14 and 16-19 weeks' gestation
Study design	Case-control
Target condition and reference standard(s)	Down's syndrome: 23 cases Reference standard: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT () Second trimester AFP, hCG and uE3 ()

Herman 2002 (*Continued*)

Follow-up	Some cases obtained through follow-up to birth. No details of follow-up in controls reported
Aim of study	To compare the results of the disclosure and non-disclosure approaches, using the clinical data of first trimester ultrasound and second trimester serum screening tests among the same groups of normal and trisomy 21-affected pregnancies
Test characteristics	
Reference standard used	
Notes	

Table of Methodological Quality ***Table of Metho***

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Yes	All women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given

Lam 2002

Clinical features and settings	Routine screening
Participants	16,237 participants undergoing NT and biochemical testing Hong Kong - multi-centre study 1997 to 2000 Pregnant women Mean maternal age 30.5 years (19% > 35 years) 10-14 weeks and 15-18 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 35 cases Reference standards: women considered high risk offered CVS (0.7%) or amniocentesis (11.8%). Follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF methods) Second trimester free ßhCG and AFP (methods not reported)
Follow-up	By review of hospital and laboratory records and by directly telephoning women. Participants who defaulted the second trimester serum tests (n = 1015) and those who miscarried after NT but before serum testing (n = 91) were excluded from the study. Outcome obtained in 15,253 patients (93.9%)
Aim of study	To report data on participants undergoing both first and second trimester methods of screening to assess the relative efficacy of different methods of screening
Test characteristics	
Reference standard used	
Notes	

Table of Methodological Quality ***Table of Metho***

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results

Lam 2002 (*Continued*)

Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	Yes	NT successful in 99.8% of cases
Withdrawals explained? All tests	Yes	Details given for patients excluded and those without follow-up data

Malone 2005

Clinical features and settings	Routine screening
Participants	38,033 participants USA - multi-centre study (15 centres) October 1999 to December 2002 Pregnant women 21.6% of women aged ≥ 35 years Singleton pregnancies Live fetuses 10-13 and 15-18 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 92 cases (87 had first trimester and second trimester screening) Reference standards: amniocentesis (offered to women with positive results from any screening test) or follow-up to birth
Index and comparator tests	Maternal age First trimester NT in 36,306 patients (92.9%) First trimester PAPP-A and free ßhCG in 37,843 patients (99.5%) Second trimester AFP, total hGC, uE3 and inhibin-A in 35,236 patients (92.6%) All tests done in 33,546 patients (88.2%)
Follow-up	Follow-up with computerised tracking system. Medical records were reviewed in cases of 1) possible medical problem suspected 2) positive screening test results with no karyotype data, 3) 10% random sample of all enrolled patients. Follow-up to birth complete in 36, 378 patients (97%)

Malone 2005 (*Continued*)

Aim of study	To evaluate first trimester and/or second trimester screening tools for Down's syndrome
Test characteristics	
Reference standard used	
Notes	Unclear which types of patients did not have follow-up data. Appears that aborted/ miscarried fetuses did not have follow-up (note in table)

Table of Methodological Quality ***Table of Metho***

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	No	Not all women received a reference standard (3% had no ascertainment of pregnancy outcome, patients not excluded from study)
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	Yes	NT failed or rejected at review in 7.1% or women
Withdrawals explained? All tests	Yes	Details given for patients who did not undergo different index tests

Okun 2008 Integrated

Clinical features and settings	Routine screening
Participants	32,227 participants undergoing integrated screening (a separate cohort evaluated for first trimester screening) January 2003 - December 2005 Canada - 2 hospitals Pregnant women Mean maternal age 32 years 11-14 and 15-18 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 86 affected cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (most sonographers had FMF certification) First trimester free ßhCG and PAPP-A (DSX Four Plate Automated ELISA Processing system, Dynex Technologies and DPC Immulite 2000 automated immunoassay analyser, Siemens Medical Solutions Diagnostics) Second trimester hCG, AFP and uE3 (Time-resolved fluoroimmunoassay, PerkinElmer AutoDelfia) Risk cut-point 1:200 or NT $\geq$ 3.5 mm Results presented with and without adjustment for bias due to miscarriages (viability bias)
Follow-up	From cytogenetics databases in both Hospitals, the Canadian Institute for Health Information, labour and delivery databases, written and phone follow-up with care providers and phone follow-up with women after birth
Aim of study	To evaluate the performance of integrated prenatal screening and first trimester combined screening for trisomy 21 in a large Canadian urban centre
Test characteristics	
Reference standard used	
Notes	

Table of Methodological Quality ***Table of Metho***

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth

Okun 2008 Integrated (*Continued*)

Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	Yes	2614 (8%) of women undergoing integrated screening did not return for the second trimester part of the test

Palomaki 2006

Clinical features and settings	Routine screening
Participants	540 participants: 32 cases and 508 controls selected from same time period (within 1 month) New York - General Hospital Singleton pregnancies Pregnant women Mean maternal age cases 33.9 years (SD 4.4 years) and controls 35.9 years (SD 3.6 years) 10-13 and 14-20 weeks' gestation
Study design	Case-control study
Target condition and reference standard(s)	Down's syndrome: 32 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age Fresh samples tested for first trimester PAPP-A and Second trimester AFP, uE3 and hCG (PerkinElmer Life and Analytical Sciences, Woodbridge, Ontario, Canada) Frozen samples thawed and tested for second trimester inhibin-A (Diagnostic Systems Laboratories, Webster, TX) and PAPP-A (PerkinElmer) Cut-points of 1:100, 1:150, 1:200 and 1:250

Palomaki 2006 (*Continued*)

Follow-up	Outcome of pregnancy available from the Ontario Multiple Marker Screening Database
Aim of study	To confirm that measuring pregnancy-associated plasma protein-A in both first and second trimester serum samples improves Down's syndrome screening
Test characteristics	
Reference standard used	
Notes	

Table of Methodological Quality ***Table of Metho***

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Yes	All women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	Yes	2 cases and 6 controls had insufficient sample to measure second trimester inhibin-A and were removed from the analysis
Withdrawals explained? All tests	No	No details of withdrawals given

Rodrigues 2009

Clinical features and settings	Routine screening
Participants	3299 participants: 2290 undergoing integrated and 1009 undergoing serum integrated screening Portugal - screening programme March 2003 - August 2007 Pregnant women Median maternal age: integrated screening 30.6 years, serum integrated screening 30.9 years First and second trimester
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 14 cases (integrated screening 8, serum integrated screening 6) Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (details not reported) First trimester PAPP-A and second trimester free ßhCG and AFP (TRACE technology, Brahms Kryptor Systems) Risk cut-point 1:300 for integrated and serum integrated screening
Follow-up	Detail of follow-up not reported
Aim of study	To report an audit of an integrated and serum integrated screening programme
Test characteristics	
Reference standard used	
Notes	

Table of Methodological Quality ***Table of Metho***

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results

Rodrigues 2009 (*Continued*)

Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given

Rozenberg 2002

Clinical features and settings	Routine screening
Participants	9118 participants France - 2 tertiary and 4 primary referral centres March 1994 - December 1997 Pregnant women Median age 30.5 years (18-37 years) Singleton pregnancies 12-14 and 14-17 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 21 cases Reference standards: amniocentesis offered to patients with NT > 3 mm or serum marker risk was >1:250. Follow-up to birth
Index and comparator tests	Maternal age First trimester NT in 98.6% of women (FMF methods) Second trimester free ßhCG (beta hCG ELISA immunoradiometric assay) and AFP (AFP ELISA immunoradiometric assay) in 91.1% of women Both NT and biochemical testing in 60.4% of women
Follow-up	Details of follow-up not reported. 3.4% of patients were lost to follow-up and were excluded from the study. This included 113 women (1.2%) with miscarriages
Aim of study	To assess the performance of combined first trimester sonographic screening and second trimester serum screening

Rozenberg 2002 (*Continued*)

Test characteristics	
Reference standard used	
Notes	Includes cost effectiveness analysis

Table of Methodological Quality ***Table of Metho***

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Yes	All women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	Yes	NT was not able to be measured in 93 women (1.5%)
Withdrawals explained? All tests	No	No details of withdrawals given

Schuchter 2001

Clinical features and settings	Routine screening
Participants	9342 participants Austria - single institution January 1994 to December 1998 Pregnant women Mean maternal age 28 years (range 15-46 years), 10.7% ≥ 35 years

Schuchter 2001 (*Continued*)

	10-13 weeks' gestation
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 19 cases Reference standards: CVS (offered to patients with first trimester NT > 3.5 mm), amniocentesis (offered to patients with first trimester NT 2.5-3.4, high risk on second trimester serum testing (> 1:250) and those > 35 years) or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (all women) (5-MHz transducer, Acuson Corp) Second trimester AFP, E2 and hGC (triple test) offered to patients not undergoing first trimester invasive testing (99.7% of women) (AMERLEX-M 2nd Trimester kits, Ortho Clinical Diagnostics)
Follow-up	Patients included in study if they were delivered in the same hospital where they were screened. It is stated that all newborns were examined for malformations by a paediatrician after delivery
Aim of study	To evaluate screening for trisomy 21 in a low risk population utilising a combination of NT measurement in the first trimester and the triple test in the second trimester
Test characteristics	
Reference standard used	
Notes	Women having miscarriages were excluded from the study

Table of Methodological Quality ***Table of Metho***

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Yes	All women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results

Schuchter 2001 (*Continued*)

Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	Details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given

Wald 2003b

Clinical features and settings	Routine screening
Participants	606 participants: 101 cases, 505 controls matched for gestation, duration of storage and centre UK and Austria - multi-centre trial September 1996 to April 2000 Pregnant women 9-13 and 14-20 weeks' gestation
Study design	Case-control study
Target condition and reference standard(s)	Down's syndrome: 101 cases Rerence standards: invasive testing (following second trimester screening) or follow-up to birth
Index and comparator tests	First trimester NT (midsagittal section, optimal magnification of thickness of translucent space between inner skin surface and fascia covering cervical spine (white black interface (outer) - black white interface (inner), 41 models of ultrasound machine, 20 minutes allotted scanning time) First trimester and second trimester serum AFP, hCG, UE3, PAPPA, free ßhCG (time resolved fluoroimmunoassay, AutoDELFIA) First trimester and second trimester inhibin A (Sandwich enzyme linked immunosorbent assay, Oxford Bioinnovation) First trimester and second trimester urinary beta core fragment, total-hCG, ITA and free ßhCG (ITA and beta core fragment, Quest diagnostics USA)
Follow-up	Follow-up by: 1) Staff at local hospitals completed a study outcome form at, or just after. delivery, 2) Study records of CVS, amniocentesis or karyotype at birth linked to information from cytogenic laboratories, 3) Study records linked to records of cases of Down's syndrome from the National Down's Syndrome Cytogenetic Register, 4) Information obtained from local obstetrical outcome records, 5) Forms sent to all women with a request to return details of the outcome of their pregnancy, 6) Individual searches in respect of women whose outcomes of pregnancy had not been obtained by any of the previous methods. 96% Birth/Karyotype full outcome documentation obtained

Wald 2003b (*Continued*)

Aim of study	To identify the most effective, safe and cost effective strategy for antenatal screening for Down's syndrome using NT, maternal serum and urine markers in the first and second trimesters of pregnancy and maternal age in various combinations
Test characteristics	
Reference standard used	
Notes	Performance of screening assessed at 17 weeks' gestation. Study tried to be non-interventional in the first trimester - second trimester testing was aimed to be used as the basis for any referral for invasive testing

Table of Methodological Quality ***Table of Metho***

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Unclear	4% of total patient cohort did not have a documented outcome of pregnancy. Unclear if any of these included in nested case-control study
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	Yes	Rates of NT failure on average 9%. Pre-10 weeks' gestation, > 33% failure rate, declined to 7% at 12 weeks
Withdrawals explained? All tests	No	No details of withdrawals given

Wald 2009

Clinical features and settings	Routine screening
Participants	14,296 participants in whom screening for all markers were measured UK - 2 Hospitals 2003 - 2007 (2004 - 2007 for 1 hospital) Pregnant women Singleton pregnancies Median maternal age 33 years (range 15-51 years), 20% ≥ 37 years 10-13 and 14-22 weeks' gestation
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 47 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (details not reported) First trimester PAPP-A (details not reported) Second trimester AFP, uE3, hCG, free ßhCG and, at one hospital, inhibin-A (details not reported) Integrated test (at 1 of the hospitals women were given the option of having only the combined test and earlier test results) Cut-point 1:150
Follow-up	Down's syndrome pregnancies, including those missed by screening, were ascertained from hospital records, cytogenetic laboratories and by linking data with the National Down Syndrome Cytogenetics Register
Aim of study	To present a medical audit of screening using the Integrated test at 2 hospitals
Test characteristics	
Reference standard used	
Notes	

Table of Methodological Quality ***Table of Metho***

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard

Wald 2009 (*Continued*)

Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given

Wright 2010 FASTER trial

Clinical features and settings	Routine screening
Participants	468 participants: 78 cases and 390 controls matched for gestational and maternal age, ethnicity and storage duration The First and Second Trimester Evaluation of Risk (FaSTER) dataset USA - 15 screening centres October 1999 - December 2002 Pregnant women Singleton pregnancies 11-13 and 15-18 weeks' gestation
Study design	Case-control study
Target condition and reference standard(s)	Down's syndrome: 78 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (details not reported) Fresh samples tested for: First trimester PAPP-A (details not reported) Second trimester AFP, uE3, hCG and inhibin A (details not reported) Frozen serum samples tested for: First trimester hCG and uE3 (details not reported) Second trimester PAPP-A (details not reported)

Wright 2010 FASTER trial (*Continued*)

	Frozen samples tested blind to other results and pregnancy outcome
Follow-up	Details not reported
Aim of study	To provide estimates and confidence intervals for the performance (detection and false positive rates) of screening for Down's syndrome using repeated measures of biochemical markers from first and second trimester maternal serum samples taken from the same woman
Test characteristics	
Reference standard used	
Notes	

Table of Methodological Quality ***Table of Metho***

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Yes	All women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given

Wright 2010 North York

Clinical features and settings	Routine screening
Participants	239 participants: 43 cases and 196 controls (35 cases and 173 controls with second trimester testing) matched for maternal and gestational age and sample date USA - The North York General Hospital dataset December 1999 - November 2007 Pregnant women Singleton pregnancies 11-13 and 14-20 weeks' gestation
Study design	Case-control study
Target condition and reference standard(s)	Down's syndrome: 43 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age Fresh samples tested for: First trimester PAPP-A (PerkinElmer) Second trimester AFP, uE3, and hCG (PerkinElmer) Frozen serum samples tested for: First trimester hCG and uE3 (details not reported) Second trimester PAPP-A (details not reported) Frozen samples tested blind to other results and pregnancy outcome
Follow-up	Details not reported
Aim of study	To provide estimates and confidence intervals for the performance (detection and false positive rates) of screening for Down's syndrome using repeated measures of biochemical markers from first and second trimester maternal serum samples taken from the same woman
Test characteristics	
Reference standard used	
Notes	

Table of Methodological Quality ***Table of Metho***

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth

Wright 2010 North York (*Continued*)

Partial verification avoided? All tests	Yes	All women received a reference standard
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given

AFP: alpha-fetoprotein
ß hCG: beta human chorionic gonadotrophin
CVS: chorionic villus sampling
ELISA: enzyme-linked immunosorbent assay
hCG: human chorionic gonadotrophin
NT: nuchal translucency
PAPP-A: pregnancy-associated plasma protein-A
SD: standard deviation
uE3: unconjugated oestriol

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Aagaard-Tillery 2010	Results presented in another study
Abbas 1995	Unable to extract useful data
Abdul-Hamid 2004	No Down's syndrome pregnancies
Abraha 1999	Unable to extract useful data

(*Continued*)

Abu-Rustum 2010	Not Down's syndrome specific
Achiron 2010	Study only includes cases of Down's syndrome
Adekunle 1999	Unable to extract useful information
Aitken 1993	Unable to extract useful data
Aitken 1996	Fewer than 80% of pregnancies had gestational age confirmed by USS
Aitken 1996a	Fewer than 80% of pregnancies had gestational age confirmed by USS
Ajayi 2011	No diagnostic data
Akbas 2001	Less than 5 Down's syndrome pregnancies
Alexioy 2009	Study only includes test positives
Allingham-Hawkins 2011	Quantitative fluorescent polymerase chain reaction study
American College 2009	Discussion article
Antona 1998	Likely fewer than 80% of pregnancies dated by USS
Antsaklis 1999	Women screened at greater than 24 weeks' gestation
Anuwutnavin 2009	Second trimester ultrasound
Ashwood 1987	Unable to extract useful data
Asrani 2005	Review article
Audibert 2001b	Unable to ascertain whether part of screening population in Rozenberg et al. No response from authors therefore excluded to reduce risk of data replication
Axt-Fleidner 2006	Unable to extract useful data
Azuma 2002	Unable to extract useful data
Baghagho 2004	Unable to obtain paper
Bahado-Singh 1995	USS markers greater than 14 weeks' gestation
Bahado-Singh 1996	USS markers greater than 14 weeks' gestation
Bahado-Singh 1999	USS markers greater than 14 weeks' gestation

(*Continued*)

Bahado-Singh 2002	USS markers greater than 14 weeks' gestation
Bahado-Singh 2003	Review article
Ball 2007	Data from the FASTER trial
Bar-Hava 2001	No Down's pregnancies in study population
Barkai 1996	No Down's pregnancies in study population
Barnabei 1995	No Down's pregnancies in study population
Bartels 1988	Unable to extract useful data
Bartels 1993	No Down's pregnancies in study population
Barth 1991	Second trimester ultrasound study
Bas-Budecka 2007	No diagnostic data
Baviera 2004	Unclear method of confirmation of gestational age
Bazzett 1998	Male versus female fetuses
Beke 2008	Results are not specific to Down's syndrome
Bellver 2005	No Down's syndrome pregnancies in study
Benn 1995	Less than 80% follow-up
Benn 1996	Less than 80% follow-up
Benn 1997	No Down's pregnancies in study population
Benn 1998	Less than 80% follow-up
Benn 2001	Statistical modelling (computer simulation)
Benn 2002	Modelled data
Benn 2003	Less than 80% of pregnancies dated by USS
Benn 2003a	Editorial
Benn 2005	No Down's pregnancies included
Benn 2005a	Mathematical model

(*Continued*)

Benn 2007	No follow-up information
Berry 1995	Less than 80% of pregnancies USS dated
Berry 1997	Less than 80% of pregnancies USS dated
Bersinger 1994	Gestational age not USS estimated
Bersinger 2000	Unable to extract useful data
Bersinger 2001	No Down's syndrome pregnancies in study population
Bersinger 2003	Unable to extract useful data
Bersinger 2004	No Down's syndrome pregnancies in study population
Bersinger 2005	No Down's syndrome pregnancies in study population
Bestwick 2008	All healthy pregnancies
Biggio 2004	Cost-effectiveness analysis
Bilardo 2011	Not a proper sample - most had elevated NT
Bindra 2002	Review article
Blundell 1999	Unable to extract useful data
Boormans 2010	Study of testing on amniocentesis samples
Boots 1989	Population risk factor calculations
Bornstein 2009a	No diagnostic data
Bornstein 2009b	No diagnostic data
Bornstein 2010	No diagnostic data
Borowski 2007	No diagnostic data
Borrell 2007	No follow-up data
Borrell 2009	Based on SURUSS (Serum, Urine and Ultrasound Screening Study) data - second trimester serum parameters not actually measured
Borruto 2002	Unable to extract useful data

(*Continued*)

Bottalico 2009	Second trimester ultrasound
Boue 1990	Review article
Bradley 1994	Screen negative population gestations not confirmed by ultrasound
Braithwaite 1996	Review article
Brambati 1995	USS screening inclusive of women greater than 14 weeks' gestation
Brambati 1996	Review article
Brizot 1995	Unable to extract useful data
Brizot 1995a	Unable to extract useful data
Brizzi 1989a	Second trimester ultrasound
Brock 1990	Unable to extract useful data
Calda 2010	No data for false positive rates
Campogrande 2001	Unable to extract useful data
Canick 1988	Unable to extract useful data
Canick 1995	Unable to extract useful data
Canini 2002	No Down's syndrome pregnancies in study population
Cans 1998	Second trimester ultrasound
Carreras 1991	Second trimester ultrasound
Caughey 2007	No diagnostic data
Cebesoy 2008	No diagnostic data
Chelli 2008	No follow-up for false negatives
Chen 1999	Review article
Chen 2002	No Down's syndrome pregnancies in study population
Chen 2004	Less than 5 Down's cases in study population
Chen 2005	Unable to extract useful data

(*Continued*)

Chen 2008	No diagnostic data
Cheng 1993	Likely that fewer than 80% of gestational age confirmed by USS
Cheng 1999	Case series No Down's syndrome pregnancies in study population
Cheng 2004a	No Down's syndrome pregnancies in study population
Cheng 2004b	No Down's syndrome pregnancies in study population
Chitayat 2002	Less than 5 Down's cases in study population
Chiu 2011	Study of maternal DNA testing
Cho 2009	Study of testing amniotic fluid
Chou 2009	Not possible to calculate specificity
Christiansen 2002	Unable to extract useful data
Christiansen 2007	Unable to extract useful data
Christiansen 2008	No diagnostic data
Chung 2000	Less than 5 Down's syndrome pregnancies in study population
CNGOF 1996	Unable to obtain translation
Cocciolone 2008	Unable to extract useful data - attempted to contact author
Cole 1996	Review article
Comas 2001	USS at greater than 14 weeks
Comas 2002a	USS at greater than 14 weeks
Comas 2002b	USS at greater than 14 weeks
Comstock 2006	Unable to extract useful data
Conde 1998	Review article
Cowans 2011	No diagnostic data
Crossley 1991	Less than 80% of pregnancies had gestational age confirmation by ultrasound

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Crossley 1993	Less than 80% of pregnancies had gestational age confirmation by ultrasound
Crossley 1996	No Down's syndrome pregnancies in study population
Crossley 2002	Adjustment factors for smokers
Cuckle 1984b	Gestational age not confirmed by USS
Cuckle 1987a	Gestational age not confirmed by USS
Cuckle 1987b	No gestational age limits given
Cuckle 1990	Paper presenting adjustment factors
Cuckle 1996a	Data modelled on 4 meta-analysed studies
Cuckle 1999b	Unable to extract useful data
Cuckle 1999c	Review article
Cullen 1990	Abnormal scans only in study population
Cusick 2004	Less than 5 Down's syndrome pregnancies in study population
Cusick 2007	ST ultrasound
Dancoine 2001	No Down's syndrome pregnancies in study population
Dane 2008	Not specific to Down's syndrome
De Biasio 2000	Unable to extract useful information
De Biasio, 1999	Unable to ascertain whether overlapping populations between several papers - attempted to contact author with no response
De Biasio, 2001	Unable to ascertain whether overlapping populations between several papers - attempted to contact author with no response
De Graaf 1991	Unable to extract useful data
De Graaf 1999	Modelled data
Del Carmen Saucedo 2009	No follow-up information
DeVore 2001	Second trimester ultrasound
Dhaifalah 2007a	Unable to obtain translation

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Dhaifalah 2007b	Unable to obtain translation
Dhallan 2007	DNA testing of blood samples from parents
Dickerson 1994	Comment
Dimaio 1987	Gestational age by USS only in screen positive population
Doran 1986	Ultrasound confirmation of gestational age performed in screen positive women only
Dreux 2008	No information for specificity
Drugan 1996a	Second trimester ultrasound
Drugan 1996b	Unable to extract useful data
Drysdale 2002	Fewer than 5 Down's syndrome pregnancies in population
Dugoff 2008	Not specific to Down's syndrome
Ebell 1999	Review article
Economides 1998	Unable to extract useful data
Erickson 2004	No Down's syndrome pregnancies in population
Evans 1996	No Down's syndrome pregnancies in population
Evans 2007	Data previously presented in another study
Falcon 2005	Unable to extract useful data
Falcon 2006	Unable to extract useful data
Ford 1998	Audit
Frishman 1997	No Down's syndrome pregnancies in population
Fukada 2000	Unable to extract useful data
Gaudry 2009	Study of karyotyping
Gebb 2009	Study only examines screen positives
Geerts 2008	Study only examines abnormal foetuses
Geipel 2010	ST ultrasound

(*Continued*)

Gekas 2009	Diagnostic data from other studies
Gekas 2011a	Diagnostic data from other studies
Gekas 2011b	Diagnostic parameters from other studies
Gerovassili 2007	No diagnostic data
Ghidini 1998	Comparison of male versus female fetuses
Goetzinger 2010	Second trimester ultrasound
Goldie 1995	Fewer than 80% of study population and gestational age confirmed by USS
Gollo 2008	Only 1 case of Down's syndrome
Gonçalves 2004	Greater than 14 weeks USS screening
Goodburn 1994	Likely that fewer than 80% of pregnancies had gestational age estimated by USS
Gorduza 2007	Study of FISH technique
Grace 2010	ST ultrasound
Grati 2010	No diagnostic data
Gray 2009	ST ultrasound
Gregor 2007	Unable to obtain translation
Gregor 2009	Unable to obtain translation
Grether 2009	Systematic review and guidelines
Grozdea 2002	Unable to extract useful data
Guo 2010	Study of fetal samples
Gyselaers 2004a	Less than 80% follow-up
Gyselaers 2004b	Less than 80% follow-up
Gyselaers 2006a	Unaffected pregnancies only
Gyselaers 2006b	Unable to extract useful data
Hackshaw 1995	No Down's syndrome pregnancies in population

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Hackshaw 2001	No Down's syndrome pregnancies in population
Haddow 1992	Less than 80% of pregnancies had gestational age confirmed by ultrasound scan
Hadzsiev 2007	Study of FISH technique
Hafner 1995	Less than 5 Down's pregnancies in study population
Hallahan 1998	Gestational age greater than 24 weeks
Han 2008	Study of findings on amniocentesis
Harper 2010	Second trimester ultrasound
Harrison 2006	Less than 80% of pregnancies had gestational age confirmed by ultrasound scan
Harry 2006	Editorial
Hayashi 1995	Unable to extract useful data
Hayashi 1996	Less than 5 Down's pregnancies in study population
Heikkila 1997	Fewer than 80% of pregnancies had gestational age confirmed by USS
Heinig 2007	No Down's syndrome data
Heinonen 1996	No Down's syndrome pregnancies in population
Herman 2000	No Down's syndrome pregnancies in study population
Herman 2003	Correlation between markers, not evaluation of screening tests
Herrou 1992	Unable to extract useful data
Hershey 1985	Gestation unclear
Hershey 1986	Gestation based on LMP
Hewitt 1993	Unable to extract useful data
Hills 2010	Study of testing on CVS and amniocentesis samples
Ho 2010	Study of FISH diagnosis
Hogdall 1992	Unclear method of determination of gestational age Unable to extract useful data

(*Continued*)

Hong Kong Practitioner	CME
Hoogendoorn 2008	Diagnostic data from other studies used
Howe 2000	Second trimester ultrasound scans
Hsiao 1991	Unable to obtain translation
Hsieh 1999	No Down's syndrome pregnancies in study population
Hsu 1997a	Adjustment factors
Hsu 1998a	No Down's syndrome pregnancies in study population
Hsu 1999b	No Down's pregnancies
Hu 2007	Same data as Liu 2010
Huang 2003	No Down's syndrome pregnancies in study population
Huang 2007a	Not possible to obtain detection rate
Huang 2007b	No diagnostic data
Huggon 2004	Study of cardiac function in pregnancies with normal and abnormal NT results
Hui 2003	No Down's syndrome pregnancies in population
Hui 2005	No Down's syndrome pregnancies in population
Hultén 2004	Editorial/commentary
Hung 2003	Modelling
Hung 2008	Second trimester ultrasound
Hurley 1993	Unable to extract useful data
Huttly 2004	No Down's syndrome pregnancies in population
Hwa 2004	Less than 5 Down's pregnancies in population
Iles 1996	Review
Ind 1994	Unable to extract useful data
Ivorra-Deleuze 2010	No diagnostic data

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Jakobsen 2011	Not Down's syndrome specific
Jean-Pierre 2005	Review article
Johnson 1991	Gestatiojnal age estimated by USS in fewer than 80% of cases
Johnson 1993	Normal pregnancies only
Jorgensen 1999	Gestation greater than 14 weeks for USS
Jorgez 2007	Study of DNA testing on maternal blood
Josefsson 1998	No Down's syndrome pregnancies in study population
Jou 2001	Less than 5 Down's syndrome pregnancies in study population
Jun-Tao 2003	Unable to obtain translation
Jung 2007	ST ultrasound
Kagan 2006	Screen positive pregnancies only
Kagan 2007	No diagnostic data
Kagan 2008	Not Down's syndrome detection
Kalelioglu 2007	ST ultrasound
Kautzmann 1995	Fewer than 80% pregnancies had gestational age estimated by USS
Kazerouni 2009	Not possible to obtain complete diagnostic data
Keith 1992	Summary article
Kelekci 2004	Less than 5 Down's syndrome pregnancies in population
Kellner 1995a	Less than 5 Down's syndrome pregnancies in population
Kellner 1995b	Less than 80% follow-up Unable to ascertain proportion of population with gestational age confirmed by USS
Kellner 1997	Assumption of normal karyotype without reference standard in significant proportion of control pregnancies
Kirkegaard 2008	FPR only calculated for subset of the cohort

(*Continued*)

Kjaergaard 2008	Unable to obtain translation
Knight 1990	Review article
Knight 2001	Validation of a specific assay
Knight 2005	Less than 80% of pregnancies had gestational age confirmed by ultrasound scan
Koos 2006	Review article
Kornman 1996	Less than 5 Down's syndrome pregnancies in population
Kornman 1997	Unable to extract useful information
Kotaska 2007	No new data
Kramer 1998	No Down's syndrome pregnancies in study population
Krantz 1996	Modelled data
Krantz 2005	Adjustment factor
Krantz 2007	Uses data from other published studies
Kulch 1993	No Down's cases in population
Lai 1998	Modelled population
Lai 2003	No Down's syndrome pregnancies in study population
Laigaard 2006a	Unable to extract useful data
Laigaard 2006b	Simulation
Lam 1997	Unable to extract useful data
Lam 1998	Fewer than 80% pregnancies had gestational age estimated by USS
Lam 1999a	No Down's syndrome pregnancies in population
Lam 1999b	Unable to extract useful data
Lam 2000	Study of women's decisions about screening
Lam 2001	Male versus female fetuses
Lambert-Messerlian 1996	Fewer than 80% of pregnancies USS dated

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Lambert-Messerlian 1998	Unable to extract useful data
Lauria 2007	No diagnostic data
Lehavi 2005	Down's syndrome pregnancies only
Leung 2006	Unable to separate twins from singletons therefore unable to extract useful data
Leymarie 1993	Appears to be a review article (French)
Li 1998	Unable to obtain translation
Li 1999	Unable to obtain translation
Li 2010	No diagnostic data
Liao 1997	Unable to obtain translation
Liao 2001	Unable to extract useful data
Lim 2002	Second trimester ultrasound
Lippman 1987	Editorial
Liu 2010	Not possible to separate out data for cases of Down's syndrome
Lo 2010	Pooled test results
Lustig 1988	Gestational age by LMP only
Luthgens 2008	FPR and DR obtained from different cohorts
MacDonald 1991	Fewer than 80% of gestational ages estimated by USS
Macintosh 1994	Unable to extract useful data
Macintosh 1997	Unable to extract useful data
MacRae 2010	Pooled test results
Macri 1994	Likely fewer than 80% evaluated for gestational age by ultrasound examination
Macri 1996	Likely fewer than 80% evaluated for gestational age by ultrasound examination
Malone 1998	Review article
Malone 2003	Review article

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Mandryka-Stankewycz 2009	No diagnostic data
Mangione 2001	Abnormal screening results only
Markov 2008	Unable to obtain paper
Maymon 2001a	No Down's syndrome pregnancies in study population
Maymon 2001b	No normal test results included therefore unable to extract meaningful data
Maymon 2002	No Down's syndrome pregnancies in study population
Maymon 2004	No Down's syndrome pregnancies in study population
Maymon 2005	Modelled data
McDuffie 1996	USS dating on screen positive women only
Meier 2002	Observed versus expected cases of Down's syndrome in a population
Merkatz 1984	Gestational age not confirmed by ultrasound scan
Merz 2005	Editorial
Merz 2008	First trimester only
Metzenbauer 2001	Normal pregnancies only
Metzenbauer 2002	Unable to extract useful data
Mikic 1999	No Down's syndrome pregnancies in study population
Miller 1991	Unable to extract useful data
Milunsky 1989	Fewer than 80% gestational age estimated by USS
Milunsky 1996	Fewer than 80% gestational age estimated by USS
Minobe 2002	Gestational age greater than specified limits
Miron 2008	No diagnostic data
Miron 2009	No diagnostic data
Miron 2010	No diagnostic data
Miyamura 1999	Unable to extract useful data

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Moghadam 1998	Unable to extract useful data
Monni 2000	Less than 5 Down's syndrome pregnancies
Monni 2002	Review article
Mooney 1994	Greater than 24 weeks' gestation
Muhcu 2008	No diagnostic data
Muller 1994	No Down's syndrome pregnancies in study population
Muller 1996	Unable to extract useful data
Muller 1999	Unable to extract useful data
Muller 2002a	Gestational age greater than 24 weeks
Muller 2002b	Unable to extract meaningful data - unable to separate double and triple test data
Muller 2003	No Down's syndrome pregnancies in study population
Murta 2002	Unable to extract useful data
Musone 2000	Unable to extract useful data
Musto 1986	Fewer than 80% USS dated
Myrick 1990	Unable to extract useful data
Naidoo 2008	Not specific Down's syndrome results
Nau 2009	No diagnostic data
Nau 2009a	No diagnostic data
Neveux 1996a	No Down's syndrome pregnancies in population
Neveux 1996b	Unable to extract useful data
Ng 2004	Unable to extract useful data
Nicolaides 1992	Study of outcomes of abnormal NT results
Nicolaides 2000	Review article
Nicolaides 2004	Review article

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Nicolaides 2005a	Unable to obtain translation - appears to be a review article
Nicolaides 2005b	Unable to obtain translation - appears to be a review article
Nicolaides 2005c	Unable to obtain translation - appears to be a review article
Nicolaides 2005d	Unable to obtain translation - appears to be a review article
Nicolaides 2005e	Unable to obtain translation - appears to be a review article
Nicolaides 2005f	Review article
Niemimaa 2001	No Down's pregnancies in study population
Niemimaa 2002	No Down's syndrome pregnancies in population
Niemimaa 2003	No Down's syndrome pregnancies in population
Noble 1997	Unable to extract useful data
Norgaard 1990	Less than 80% of gestational ages confirmed by USS
Norton 1992	Unable to extract useful data
Novakov-Mikic 2007	Out of FT screening time frame
O'Brien 1997a	No Down's syndrome pregnancies in population
O'Brien 1997b	No Down's syndrome pregnancies in population
Odibo 2004	Gestational age of greater than 14 weeks in USS population
Odibo 2007	ST ultrasound
Odibo 2008	ST ultrasound
Odibo 2009	No results presented
Offerdal 2008	ST ultrasound
Ognibene 1999	Unable to extract useful data
Oh 2007	No diagnostic data
Olajide 1989	Unable to extract useful data
Onda 1996	Unable to extract useful data

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Onda 1998	Unable to extract useful data
Onda 2000	Less than 80% follow-up
Orlandi 2002	No Down's syndrome pregnancies in study population
Ottavio 1997	Second trimester USS
Ozkaya 2010	Only healthy pregnancies
Paladini 2007	No diagnostic data
Palka 1998	Twin data used in calculation of the median
Palomaki 1989	Fewer than 80% USS dated
Palomaki 1993	No Down's syndrome pregnancies in population
Palomaki 1994	No Down's syndrome pregnancies in population
Palomaki 1996	Meta-analysis
Palomaki 2005	Unable to extract meaningful data
Panburana 2001	Less than 5 Down's syndrome pregnancies in population
Pandya 1994	Study of outcomes of abnormal NT results
Pandya 1995b	Review article
Papadopoulou 2008	No diagnostic data
Parra-Cordero 2007	ST ultrasound
Paterlini-Brechot 2007	Editorial, no new data
Paul 2001	Unable to extract useful data
Peralta 2005	Unable to extract useful data
Perenc 1998	No Down's syndrome pregnancies in study population
Perheentupa 2002	No Down's syndrome pregnancies in population
Perona 1998	Smokers versus non smokers
Persico 2008	ST ultrasound

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Petervari 2000	Unable to extract useful data
Petrocik 1989	Likely fewer than 80% USS dated
Phillips 1992	Gestational age confirmed by USS in less than 80% of population
Phillips 1993	Gestational age confirmed by USS in less than 80% of population
Pihl 2008	Only 2 cases of Down's syndrome
Pinette 2003	Women screened prior to recruitment
Platt 2004	Unable to extract useful data
Podobnik 1995	Abnormal results only
Poon 2009	No diagnostic data
Prefumo 2002	Comparison of prevalence and prediction
Prefumo 2004	Comparison of a marker in women of different ethnic origins
Price 1998	Unable to extract useful data
Páez 2004	Unable to obtain translation
Rembouskos 2004	Unable to extract useful data
Ren 1992	Review article
Renier 1998	Method of ascertainment of gestational age unclear Twin gestations included in general population
Resta 1990	Second trimester USS
Reynders 1997	Fewer than 5 Down's cases
Reynolds 1989	Explanation of mathematical techniques
Reynolds 1999	Unable to extract useful data
Reynolds 2008	Not full diagnostic data
Ribbert 1996	No Down's syndrome pregnancies in study population
Rice 2005	Down's syndrome pregnancies excluded from study
Rich 1991	Unable to extract useful data

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Roberts 1995	No Down's syndrome pregnancies in study population
Robertson 1991	Editorial
Rode 2003	No Down's pregnancies
Ronge 2006	Editorial - summary of FASTER results
Rose 1995	Review article
Ross 1997	Review article
Rotmensch 1996	Unable to extract useful data
Rotmensch 1999	No Down's syndrome pregnancies in study population
Rozenberg 2006	USS greater than 14 weeks' gestation
Rudnicka 2002	No Down's syndrome pregnancies in population
Ryall 1992	Unable to determine method of confirmation of gestational age
Ryall 2001	High-risk results only included (i.e. no screen negative group for comparison)
Räty 2000	No Down's syndrome pregnancies in population
Räty 2002	No Down's pregnancies in population
Sabriá 2002	Unable to ascertain how numbers calculated and from which populations
Sacchini 2003	Unable to extract useful data
Sahota 2009	No diagnostic data
Sahota 2010	Included in Sahota 2010
Salazar 2007	Unable to obtain paper
Salazar 2008	Only 1 case of Down's syndrome
Saller 1997	Down's syndrome secondary to Robertsonian translocation only. No controls
Salomon 2001	No Down's syndrome pregnancies in population
Salonen 1997	Fewer than 80% had gestational age estimated by USS
Saltvedt 2005	Gestation greater than 14 weeks for nuchal scanning

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Saridogan 1996	Down's syndrome and Edward's syndrome affected pregnancies only
Savoldelli 1993	Unable to extract useful data
Schielen 2009	Full study information not given
Schiott 2006	Unable to extract useful data
Schmidt 2007a	Not specific to Down's syndrome
Schmidt 2007b	No separate Down's syndrome data
Schmidt 2007c	No diagnostic data
Schmidt 2008a	Not specific to Down's syndrome
Schmidt 2008b	Not specific to Down's syndrome
Schmidt 2008c	Not specific to Down's syndrome
Schmidt 2010	No follow-up data for test negatives
Schuchter 1998	No Down's pregnancies in study population
Scott 1995	Less than 5 Down's syndrome pregnancies in study population
Seeds 1990	Review article
Seki 1995	No Down's syndrome pregnancies in study population
Shenhav 2003	No Down's syndrome pregnancies
Shintaku 1989	Unable to extract useful data
Shulman 2003	No Down's syndrome pregnancies in population
Sieroszewski 2008	No Down's syndrome specific information for specificity
Simon-Bouy 1999	Review article
Simpson 1986	Gestational age confirmed by USS in less than 80% of population
Smith 1990	Analysis of screen positive results
Smith 1996	Review/meta-analysis
Smith 1999	Unable to extract useful data

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Smith-Bindman 2001	Meta-analysis of second trimester ultrasound markers
Smith-Bindman 2003	Population study, not examining DTA
Snijders 1995	Study of prevalence, not screening
Snijders 1999	Study of prevalence, not screening
Soergel 2006	Less than 80% follow-up
Sokol 1998	Observation of Down's prevalence stratified by age
Sonek 2003	Editorial
Sonek 2007	ST ultrasound
Sood 2010	No diagnostic data
Sooklim 2010	ST ultrasound
Spencer 1985	Fewer than 80% USS dated
Spencer 1991a	Likely fewer than 80% USS dated
Spencer 1991b	Unable to extract useful data
Spencer 1992	Unable to extract useful data
Spencer 1993a	Fewer than 80% USS dated
Spencer 1993b	No Down's pregnancies in study population
Spencer 1993c	Unable to extract useful data
Spencer 1993d	Fewer than 80% of pregnancies had gestational age confirmed by USS
Spencer 1993e	Unable to extract useful data
Spencer 1995	No Down's pregnancies in population
Spencer 1996	Fewer than 80% of pregnancies had gestational age confirmed by USS
Spencer 1997	Statistical modelling, aneuploid pregnancies only in study population
Spencer 1998a	No Down's pregnancies in population
Spencer 1998b	Unable to extract useful data

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Spencer 1999a	Review
Spencer 1999b	Statistical methods paper
Spencer 2000a	Examination of median shifts rather than an evaluation of screening
Spencer 2000b	No Down's syndrome pregnancies in population
Spencer 2000c	No Down's syndrome pregnancies in population
Spencer 2000d	No Down's cases
Spencer 2000e	Male versus female fetuses
Spencer 2000f	No Down's cases in population
Spencer 2000g	No Down's pregnancies in population
Spencer 2000h	No Down's pregnancies in population
Spencer 2000i	Comparsison of fetal sex
Spencer 2001a	No Down's syndrome pregnancies in population
Spencer 2001b	Unable to extract useful data
Spencer 2001c	Unable to extract useful data
Spencer 2001d	Unable to extract useful data
Spencer 2001e	No Down's syndrome pregnancies in population
Spencer 2002a	No Down's pregnancies
Spencer 2002b	Risk validation study
Spencer 2002c	No Down's syndrome pregnancies in population
Spencer 2002d	Demonstration of median changes with time, rather than evaluation of screening
Spencer 2003a	No Down's pregnancies in population
Spencer 2003b	No Down's pregnancies in population
Spencer 2003c	Calculation of weight correction factor

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Spencer 2003d	Fewer than 5 Down's syndrome pregnancies
Spencer 2004	Calculation of smoking correction factor
Spencer 2005a	No Down's pregnancies
Spencer 2005b	No Down's pregnancies
Spencer 2005c	Comparison of two different assays - not actual screening evaluation
Spencer 2008b	Unable to extract appropriate data for unaffected pregnancies
Spong 1999	Comparison of male and female fetuses
Staboulidou 2009	No diagnostic data
Stevens 1998	Literature review
Stoll 1992	Review article
Stressig 2011	ST ultrasound
Su 2002	Unable to extract useful data
Suchet 1995	Review article
Suchy 1990	Unable to ascertain method of confirmation of gestational age
Summers 2003a	Only 55% gestational ages estimated by USS
Summers 2003b	No Down's syndrome pregnancies in study population
Suntharasaj 2005	Examination of inter-observer variation in NT scanning
Susman 2010	No diagnostic data
Sutton 2004	Unable to extract useful data
Suzuki 1998	Unable to extract useful data
Tabor 1987	Geststional age not confirmed by USS
Tanski 1999	Information on screen positive pregnancies only
Thilaganathan 1998	No Down's syndrome pregnancies in study population
Thilaganathan 1999	Editorial

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Tislaric 2002	No Down's syndrome pregnancies in population
Torok 1997	Unable to extract useful data
Torring 2009	Not possible to obtain full diagnostic data
Trninic-Pjevic 2007	Unable to obtain translation
Tsai 2001	Less than 5 Down's syndrome pregnancies in study population
Valerio 1996	Fewer than 80% pregnancies had gestational age estimated by USS
Van Blerk 1992	Unable to extract useful data
Van Dyke 2007	Not possible to obtain full diagnostic data
Van Heesch, 2006	No Down's syndrome pregnancies in study population Software comparison study
Van Lith 1991	Unable to extract useful data
Van Lith 1993	Unable to extract useful data
Van Lith 1994	Unable to extract useful data
Veress 1986	Unable to extract useful data
Veress 1988	Unable to extract useful data
Vergani 2008	ST ultrasound
Vintzileos 2003	Second trimester USS
Wald 1988a	Less than 80% had gestational age confirmed by ultrasound
Wald 1988b	Gestational age not confirmed by USS
Wald 1991	No Down's pregnancies in study
Wald 1992a	Less than 80% had gestational age confirmed by ultrasound
Wald 1992b	No Down's pregnancies in study
Wald 1992c	No Down's pregnancies in study
Wald 1993	No USS dating

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Wald 1994a	No Down's syndrome pregnancies in population
Wald 1994b	Review article
Wald 1996a	No Down's pregnancies
Wald 1996b	Dated by LMP
Wald 1996c	No Down's syndrome pregnancies in population
Wald 1996d	Gestational age greater than 24 weeks
Wald 1997	Data modelled on 3 separate populations of women
Wald 1998	Unable to extract useful data
Wald 1999a	Unable to extract useful data
Wald 1999b	Gestational age not confirmed by USS
Wald 1999c	No Down's syndrome pregnancies
Wald 1999d	Modelled on several studies, some of which have no USS dating
Wald 2003c	No cases
Wald 2003d	Less than 80% had gestational age confirmed by USS
Wald 2006	Modelled on SURRUS data
Wallace 1994	Unable to extract useful data
Wallace 1997	No Down's syndrome pregnancies in study population
Wang 2010	ST ultrasound
Ward 2005	Review article
Watt 1996	No Down's syndrome pregnancies in study population
Watt 1996a	No Down's syndrome pregnancies in study population
Wax 2007	No diagnostic data
Weinans 2001	Unable to extract useful data
Weinans 2004	Study of women's views on screening

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Weisz 2007	Cohort split into people having different tests and non-representative samples of women assessed for each test
Welborn 1994	Abnormal results only (cystic hygroma)
Wenstrom 1993	Less than 80% of pregnancies had gestational age confirmed by USS
Wenstrom 1995a	Adjustment factors
Wenstrom 1995b	Less than 80% of pregnancies had gestational age confirmed by USS
Wetta 2011	No diagnostic data
Whitlow 1998a	Unable to extract useful data
Whitlow 1998b	Unable to extract useful data
Whitlow 1999	Unable to extract useful data
Williamson 1994	Likely fewer than 80% USS dated
Wilson 2000	Review
Wojdemann 2001	No Down's syndrome pregnancies in study population
Wong 2003	Less than 5 Down's syndrome pregnancies in population
Wright 2006	Mathematical model
Wright 2007	Simulation study, no new data
Xie 2010	Only cases of false negatives and true negatives included
Yagel 1998	Second trimester USS
Yamamoto 2001a	Unable to extract useful data
Yamamoto 2001b	Method of determination of gestational age unclear
Yamamoto 2001c	Unable to extract useful data
Yaron 2001	Male versus female fetuses
Ye 1995	Unable to obtain translation
Yoshida 2000	Fewer than 80% pregnancies had gestational age estimated by USS
Zalel 2008	No diagnostic data

(*Continued*)

Zeitune 1991	Only aneuploid pregnancies included in study
Zelop 2005	No Down's cases in population
Zhang 2011	No diagnostic data
Zhao 1998	Unable to obtain translation
Zhong 2011	Second trimester ultrasound
Zoppi 2003	Inappropriate study design

CVS: CVS: chorionic villus sampling
FISH: Fluorescence in situ hybridisation
FPR: false positive rate
LMP: last menstrual period
NT: nuchal transparency
SURUSS: Serum, Urine and Ultrasound Screening Study
USS: ultrasound screening

DATA

Presented below are all the data for all of the tests entered into the review.

Tests. Data tables by test

Test	No. of studies	No. of participants
1 Age, 1T PAPP-A , 2T free ßhCG and 2T AFP at 5% FPR	1	1188
2 Age, 1T PAPP-A , 2T free ßhCG and 2T AFP, risk 1:300	1	1009
3 Age, 1T PAPP-A , 2T total hCG, and 2T AFP at 5% FPR	1	1188
4 Age, 1T PAPP-A , 2T free ßhCG, 2T uE3 and 2T AFP at 5% FPR	1	1188
5 Age, 1T PAPP-A , 2T total hCG, 2T uE3 and 2T AFP at 2% FPR	2	707
6 Age, 1T PAPP-A , 2T total hCG, 2T uE3 and 2T AFP at 5% FPR	2	1767
7 Age, 1T PAPP-A , 2T total hCG, 2T uE3 and 2T AFP at risk 1:200	2	707
8 Age, 1T PAPP-A , 2T total hCG, 2T uE3 and 2T AFP, mixed cutpoints	4	2474
9 Age, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A at 5% FPR	1	1188
10 Age, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:50	1	1188
11 Age, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:100	1	1188
12 Age, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:150	1	1188
13 Age, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:200	1	1188
14 Age, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:250	1	1188

15 Age, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:300	1	1188
16 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A at 5% FPR	2	34821
17 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:100	1	540
18 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:150	1	540
19 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:200	1	540
20 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:250	1	540
21 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A, mixed cutpoints	3	35361
22 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T PAPP-A at 2% FPR	2	707
23 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T PAPP-A at risk 1:200	2	707
24 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP, 2T Inhibin A and 2T PAPP-A at risk 1:100	1	540
25 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP, 2T Inhibin A and 2T PAPP-A at risk 1:150	1	540
26 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP, 2T Inhibin A and 2T PAPP-A at risk 1:200	1	540
27 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP, 2T Inhibin A and 2T PAPP-A at risk 1:250	1	540
28 Age, 1T PAPP-A , 1T total hCG, 2T total hCG, 2T uE3 and 2T AFP at 2% FPR	2	707
29 Age, 1T PAPP-A , 1T total hCG, 2T total hCG, 2T uE3 and 2T AFP at risk 1:200	2	707

30 Age, 1T PAPP-A , 1T uE3, 2T total hCG, 2T uE3 and 2T AFP at 2% FPR	2	707
31 Age, 1T PAPP-A , 1T uE3, 2T total hCG, 2T uE3 and 2T AFP at risk 1:200	2	707
32 Age, 1T PAPP-A , 1T total hCG, 1T uE3, 2T total hCG, 2T uE3, 2T AFP and 2T PAPP-A at 2% FPR	2	707
33 Age, 1T PAPP-A , 1T total hCG, 1T uE3, 2T total hCG, 2T uE3, 2T AFP and 2T PAPP-A at risk 1:200	2	707
34 Age, 1T AFP, 1T free ßhCG, 1T uE3, 2T total hCG, 2T uE3 and 2T AFP at risk 1:250	1	12339
35 Age, 1T AFP, 1T free ßhCG, 1T uE3, 2T total hCG, 2T uE3 and 2T AFP at risk 1:384	1	12339
36 Age, 1T NT, 2T total hCG and 2T AFP, 5FPR	2	17347
37 Age, 1T NT, 2T total hCG and 2T AFP, risk 1:250	2	5446
38 Age, 1T NT, 2T total hCG and 2T AFP, mixture cutpoint	4	22793
39 Age, 1T NT, 2T free ßhCG and 2T AFP, 5FPR	2	6616
40 Age, 1T NT, 2T free ßhCG and 2T AFP, mixture cutpoint	2	6616
41 Age, 1T NT, 2T free ßhCG, 2T uE3 and 2T AFP, 5FPR	1	1110
42 Age, 1T NT, 2T total hCG, 2T uE3 and 2T AFP, 5FPR	1	1110
43 Age, 1T NT, 2T total hCG, 2T uE3 and 2T AFP, risk 1:250	2	3256
44 Age, 1T NT, 2T total hCG, 2T uE3 and 2T AFP, mixture cutpoint	4	13708
45 Age, 1T NT, 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A, 5FPR	1	1110
46 Age, 1T NT, 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A, 5FPR	1	1110
47 Age, 1T NT, 2T free ßhCG, 2T uE3, 2T AFP and 1T PAPP-A , 5FPR	1	1110
48 Age, 1T NT, 2T free ßhCG, 2T uE3, 2T AFP and 1T PAPP-A , risk 1:250	1	390

49 Age, 1T NT, 1T PAPP-A , 2T total hCG and 2T AFP, 5FPR	1	1110
50 Age, 1T NT, 1T PAPP-A , 2T free ßhCG and 2T AFP, 5FPR	1	1110
51 Age, 1T NT, 1T PAPP-A , 2T free ßhCG and 2T AFP,risk 1:250	1	390
52 Age, 1T NT, 1T PAPP-A , 2T free ßhCG and 2T AFP, risk 1:300	1	2290
53 Age, 1T NT, 1T PAPP-A, 2T total hCG, 2T uE3 and 2T AFP 5FPR	1	1110
54 Age, 1T NT, 1T PAPP-A , 2T total hCG, 2T uE3 and 2T AFP, risk 1:200	1	32227
55 Age, 1T NT, 1T PAPP-A, 2T total hCG, 2T uE3 and 2T AFP, mixed cutpoints	2	33337
56 Age, 1T NT, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A, 5FPR	2	34743
57 Age, 1T NT, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A, risk 1:150	1	4927
58 Age, 1T NT, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A, mixed cutpoints	3	39670
59 Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:300	1	390
60 Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A, 1:270	1	7842
61 Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:250	1	390
62 Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:200	1	390
63 Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:150	2	9759
64 Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:100	1	390
65 Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:50	1	390

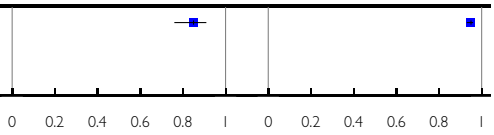
66 Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A, 5FPR	3	31698
67 Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A, 3FPR	1	22746
68 Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A, 1FPR	1	22746
69 Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A, mixed cutpoints	4	40348
70 Age, 1T NT, 1T PAPP-A, 1T free ßhCG, 2T total hCG, 2T uE3 and 2T AFP, risk 1:250	1	5060
71 Age, 1T NT, 1T PAPP-A, 1T free ßhCG, 2T uE3, 2T AFP, 2T total hCG and 2T Inhibin A, risk 1:150	1	33546
72 ADAM 12 2T TO 1T RATIO	1	579
73 Stepwise: Age, 1T NT, 1T PAPP-A , 1T free ßhCG, if risk <1/30, 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A risk 1:270	1	32355
74 Stepwise: Age, 1T NT, 1T PAPP-A , 1T free ßhCG, if risk <1/30, 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A risk 1:270	1	7842
75 Stepwise: Age, 1T NT, 1T PAPP-A , 1T free ßhCG, if risk <1/30, 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A 5% FPR	1	7842
76 Stepwise: Age, 1T NT, 1T PAPP-A , if risk <1:100, 2T free ßhCG, 2T uE3, 2T AFP, risk 1:250	1	1507
77 Contingent: Age, 1T NT, 1T PAPP-A , 1T free ßhCG, if risk 1/30-1/1500, 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A risk 1:270	1	32355
78 Contingent: Age, 1T NT, 1T PAPP-A , 1T free ßhCG, if risk 1/30-1/1500, 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A risk 1:270	1	7842

Test 1. Age, 1T PAPP-A , 2T free ßhCG and 2T AFP at 5% FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 1 Age, 1T PAPP-A , 2T free hCG and 2T AFP at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003b	83	55	15	1035	0.85 [0.76, 0.91]	0.95 [0.93, 0.96]		

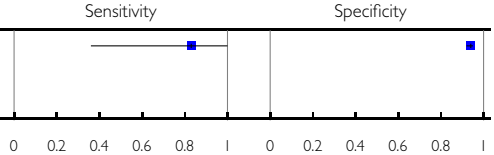


Test 2. Age, 1T PAPP-A , 2T free ßhCG and 2T AFP, risk 1:300.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 2 Age, 1T PAPP-A , 2T free hCG and 2T AFP, risk 1:300

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Rodrigues 2009	5	60	1	943	0.83 [0.36, 1.00]	0.94 [0.92, 0.95]		

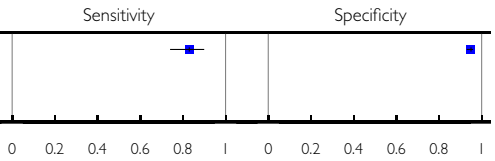


Test 3. Age, 1T PAPP-A , 2T total hCG, and 2T AFP at 5% FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 3 Age, 1T PAPP-A , 2T total hCG, and 2T AFP at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003b	81	55	17	1035	0.83 [0.74, 0.90]	0.95 [0.93, 0.96]

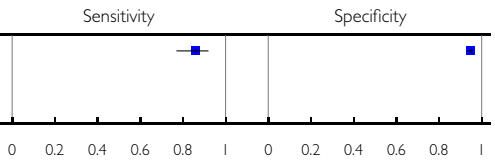


Test 4. Age, 1T PAPP-A , 2T free ßhCG, 2T uE3 and 2T AFP at 5% FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 4 Age, 1T PAPP-A , 2T free hCG, 2T uE3 and 2T AFP at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003b	84	55	14	1035	0.86 [0.77, 0.92]	0.95 [0.93, 0.96]

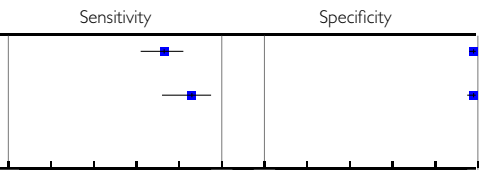


Test 5. Age, 1T PAPP-A , 2T total hCG, 2T uE3 and 2T AFP at 2% FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 5 Age, 1T PAPP-A , 2T total hCG, 2T uE3 and 2T AFP at 2% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wright 2010 FASTER trial	57	8	21	382	0.73 [0.62, 0.82]	0.98 [0.96, 0.99]
Wright 2010 North York	37	4	6	192	0.86 [0.72, 0.95]	0.98 [0.95, 0.99]

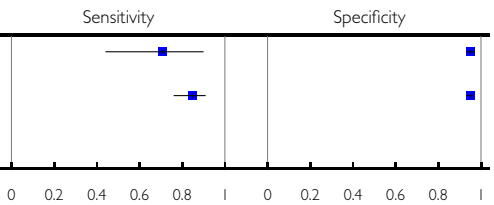


Test 6. Age, 1T PAPP-A , 2T total hCG, 2T uE3 and 2T AFP at 5% FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 6 Age, 1T PAPP-A , 2T total hCG, 2T uE3 and 2T AFP at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Baviera 2010	12	28	5	534	0.71 [0.44, 0.90]	0.95 [0.93, 0.97]
Wald 2003b	83	55	15	1035	0.85 [0.76, 0.91]	0.95 [0.93, 0.96]

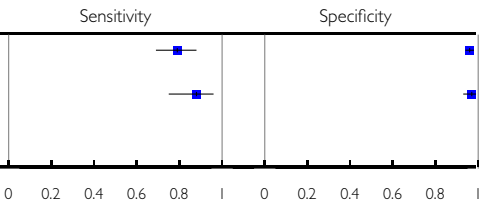


Test 7. Age, 1T PAPP-A , 2T total hCG, 2T uE3 and 2T AFP at risk 1:200.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 7 Age, 1T PAPP-A , 2T total hCG, 2T uE3 and 2T AFP at risk 1:200

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wright 2010 FASTER trial	62	14	16	376	0.79 [0.69, 0.88]	0.96 [0.94, 0.98]
Wright 2010 North York	38	6	5	190	0.88 [0.75, 0.96]	0.97 [0.93, 0.99]

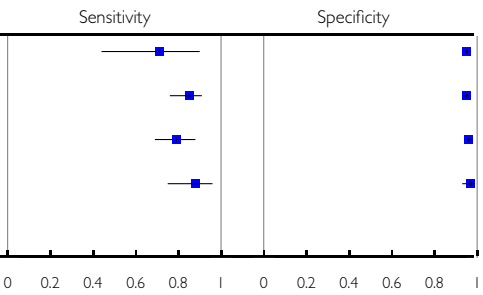


Test 8. Age, 1T PAPP-A , 2T total hCG, 2T uE3 and 2T AFP, mixed cutpoints.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 8 Age, 1T PAPP-A , 2T total hCG, 2T uE3 and 2T AFP, mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Baviera 2010	12	28	5	534	0.71 [0.44, 0.90]	0.95 [0.93, 0.97]
Wald 2003b	83	55	15	1035	0.85 [0.76, 0.91]	0.95 [0.93, 0.96]
Wright 2010 FASTER trial	62	14	16	376	0.79 [0.69, 0.88]	0.96 [0.94, 0.98]
Wright 2010 North York	38	6	5	190	0.88 [0.75, 0.96]	0.97 [0.93, 0.99]

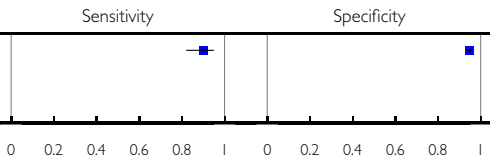


Test 9. Age, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A at 5% FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 9 Age, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003b	88	55	10	1035	0.90 [0.82, 0.95]	0.95 [0.93, 0.96]

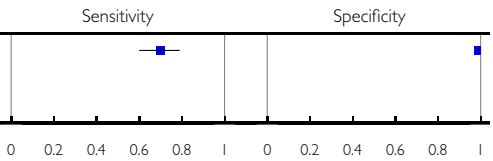


Test 10. Age, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:50.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 10 Age, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:50

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003b	69	12	29	1078	0.70 [0.60, 0.79]	0.99 [0.98, 0.99]

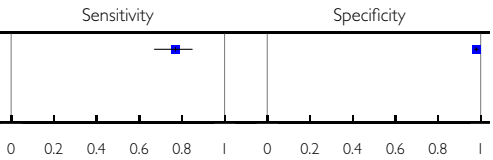


Test 11. Age, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:100.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 11 Age, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:100

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003b	75	24	23	1066	0.77 [0.67, 0.85]	0.98 [0.97, 0.99]

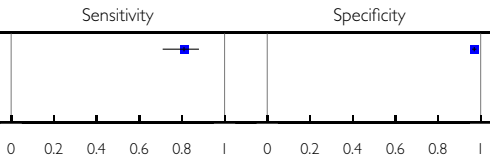


Test 12. Age, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:150.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 12 Age, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:150

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003b	79	35	19	1055	0.81 [0.71, 0.88]	0.97 [0.96, 0.98]



Test 13. Age, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:200.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 13 Age, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:200

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003b	81	45	17	1045	0.83 [0.74, 0.90]	0.96 [0.95, 0.97]		

Test 14. Age, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:250.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 14 Age, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:250

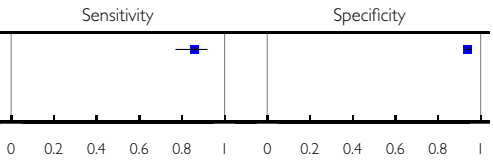
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003b	83	55	15	1035	0.85 [0.76, 0.91]	0.95 [0.93, 0.96]		

Test 15. Age, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:300.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 15 Age, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:300

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003b	84	63	14	1027	0.86 [0.77, 0.92]	0.94 [0.93, 0.96]

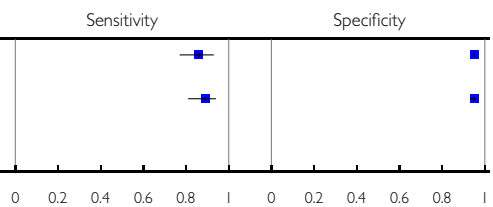


Test 16. Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A at 5% FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 16 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Malone 2005	75	1677	12	31869	0.86 [0.77, 0.93]	0.95 [0.95, 0.95]
Wald 2003b	87	55	11	1035	0.89 [0.81, 0.94]	0.95 [0.93, 0.96]

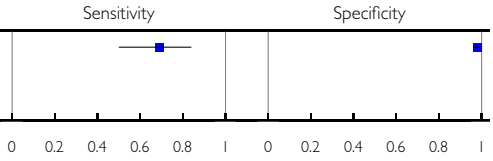


Test 17. Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:100.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 17 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:100

Study	TP	FP	FN	TN	Sensitivity	Specificity
Palomaki 2006	22	10	10	498	0.69 [0.50, 0.84]	0.98 [0.96, 0.99]



Test 18. Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:150.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 18 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:150

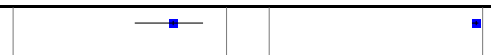
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Palomaki 2006	24	14	8	494	0.75 [0.57, 0.89]	0.97 [0.95, 0.98]		

Test 19. Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:200.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 19 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:200

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Palomaki 2006	24	17	8	491	0.75 [0.57, 0.89]	0.97 [0.95, 0.98]		

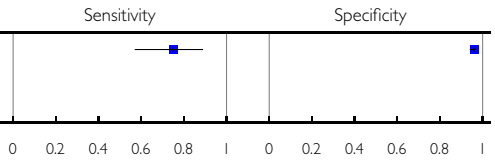


Test 20. Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:250.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 20 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A at risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity
Palomaki 2006	24	21	8	487	0.75 [0.57, 0.89]	0.96 [0.94, 0.97]

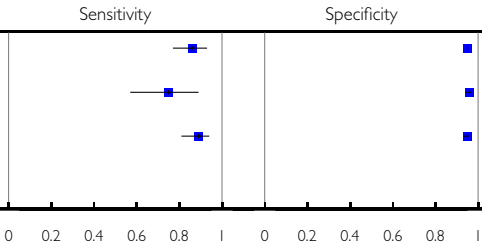


Test 21. Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A, mixed cutpoints.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 21 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A, mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Malone 2005	75	1677	12	31869	0.86 [0.77, 0.93]	0.95 [0.95, 0.95]
Palomaki 2006	24	21	8	487	0.75 [0.57, 0.89]	0.96 [0.94, 0.97]
Wald 2003b	87	55	11	1035	0.89 [0.81, 0.94]	0.95 [0.93, 0.96]

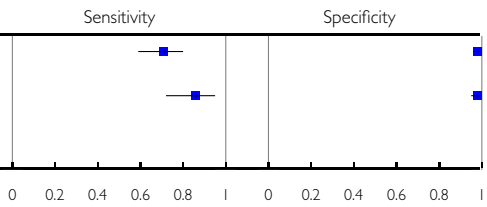


Test 22. Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T PAPP-A at 2% FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 22 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T PAPP-A at 2% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wright 2010 FASTER trial	55	8	23	382	0.71 [0.59, 0.80]	0.98 [0.96, 0.99]
Wright 2010 North York	37	4	6	192	0.86 [0.72, 0.95]	0.98 [0.95, 0.99]

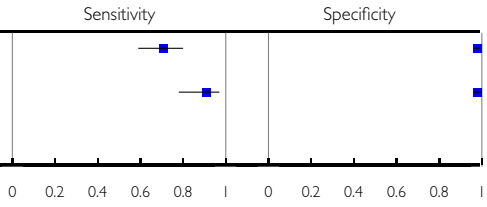


Test 23. Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T PAPP-A at risk 1:200.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 23 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T PAPP-A at risk 1:200

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wright 2010 FASTER trial	55	9	23	381	0.71 [0.59, 0.80]	0.98 [0.96, 0.99]
Wright 2010 North York	39	3	4	193	0.91 [0.78, 0.97]	0.98 [0.96, 1.00]



Test 24. Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP, 2T Inhibin A and 2T PAPP-A at risk 1:100.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 24 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP, 2T Inhibin A and 2T PAPP-A at risk 1:100

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Palomaki 2006	24	7	8	501	0.75 [0.57, 0.89]	0.99 [0.97, 0.99]		

Test 25. Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP, 2T Inhibin A and 2T PAPP-A at risk 1:150.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 25 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP, 2T Inhibin A and 2T PAPP-A at risk 1:150

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Palomaki 2006	25	11	7	497	0.78 [0.60, 0.91]	0.98 [0.96, 0.99]		

Test 26. Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP, 2T Inhibin A and 2T PAPP-A at risk 1:200.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 26 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP, 2T Inhibin A and 2T PAPP-A at risk 1:200

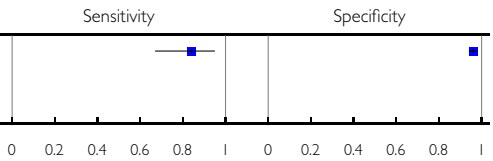
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Palomaki 2006	26	17	6	491	0.81 [0.64, 0.93]	0.97 [0.95, 0.98]		

Test 27. Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP, 2T Inhibin A and 2T PAPP-A at risk 1:250.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 27 Age, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP, 2T Inhibin A and 2T PAPP-A at risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity
Palomaki 2006	27	19	5	489	0.84 [0.67, 0.95]	0.96 [0.94, 0.98]

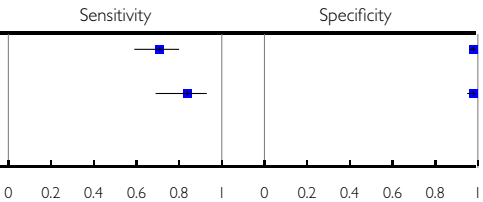


Test 28. Age, 1T PAPP-A , 1T total hCG, 2T total hCG, 2T uE3 and 2T AFP at 2% FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 28 Age, 1T PAPP-A , 1T total hCG, 2T total hCG, 2T uE3 and 2T AFP at 2% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wright 2010 FASTER trial	55	8	23	382	0.71 [0.59, 0.80]	0.98 [0.96, 0.99]
Wright 2010 North York	36	4	7	192	0.84 [0.69, 0.93]	0.98 [0.95, 0.99]

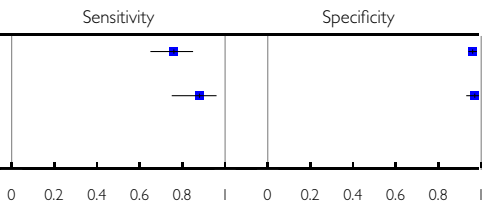


Test 29. Age, 1T PAPP-A , 1T total hCG, 2T total hCG, 2T uE3 and 2T AFP at risk 1:200.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 29 Age, 1T PAPP-A , 1T total hCG, 2T total hCG, 2T uE3 and 2T AFP at risk 1:200

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wright 2010 FASTER trial	59	14	19	376	0.76 [0.65, 0.85]	0.96 [0.94, 0.98]
Wright 2010 North York	38	6	5	190	0.88 [0.75, 0.96]	0.97 [0.93, 0.99]

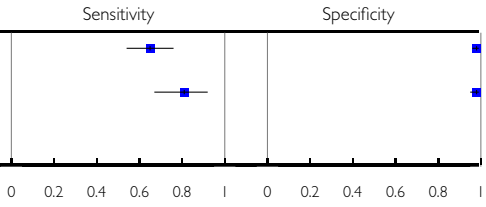


Test 30. Age, 1T PAPP-A , 1T uE3, 2T total hCG, 2T uE3 and 2T AFP at 2% FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 30 Age, 1T PAPP-A , 1T uE3, 2T total hCG, 2T uE3 and 2T AFP at 2% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wright 2010 FASTER trial	51	8	27	382	0.65 [0.54, 0.76]	0.98 [0.96, 0.99]
Wright 2010 North York	35	4	8	192	0.81 [0.67, 0.92]	0.98 [0.95, 0.99]

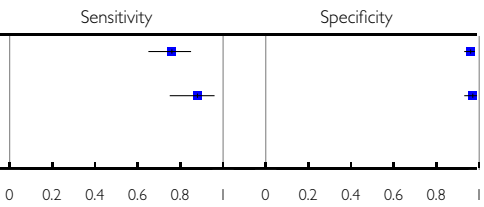


Test 31. Age, 1T PAPP-A , 1T uE3, 2T total hCG, 2T uE3 and 2T AFP at risk 1:200.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 31 Age, 1T PAPP-A , 1T uE3, 2T total hCG, 2T uE3 and 2T AFP at risk 1:200

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wright 2010 FASTER trial	59	16	19	374	0.76 [0.65, 0.85]	0.96 [0.93, 0.98]
Wright 2010 North York	38	6	5	190	0.88 [0.75, 0.96]	0.97 [0.93, 0.99]

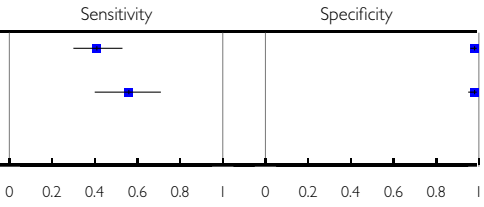


Test 32. Age, 1T PAPP-A , 1T total hCG, 1T uE3, 2T total hCG, 2T uE3, 2T AFP and 2T PAPP-A at 2% FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 32 Age, 1T PAPP-A , 1T total hCG, 1T uE3, 2T total hCG, 2T uE3, 2T AFP and 2T PAPP-A at 2% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wright 2010 FASTER trial	32	8	46	382	0.41 [0.30, 0.53]	0.98 [0.96, 0.99]
Wright 2010 North York	24	4	19	192	0.56 [0.40, 0.71]	0.98 [0.95, 0.99]

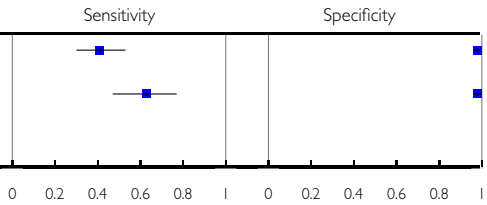


Test 33. Age, 1T PAPP-A , 1T total hCG, 1T uE3, 2T total hCG, 2T uE3, 2T AFP and 2T PAPP-A at risk 1:200.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 33 Age, 1T PAPP-A , 1T total hCG, 1T uE3, 2T total hCG, 2T uE3, 2T AFP and 2T PAPP-A at risk 1:200

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wright 2010 FASTER trial	32	7	46	383	0.41 [0.30, 0.53]	0.98 [0.96, 0.99]
Wright 2010 North York	27	3	16	193	0.63 [0.47, 0.77]	0.98 [0.96, 1.00]

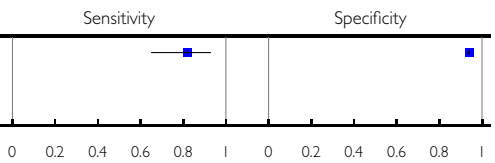


Test 34. Age, 1T AFP, 1T free ßhCG, 1T uE3, 2T total hCG, 2T uE3 and 2T AFP at risk 1:250.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 34 Age, 1T AFP, 1T free hCG, 1T uE3, 2T total hCG, 2T uE3 and 2T AFP at risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity
Goh 1996	28	773	6	11532	0.82 [0.65, 0.93]	0.94 [0.93, 0.94]

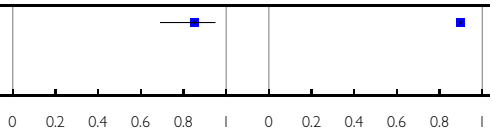


Test 35. Age, 1T AFP, 1T free ßhCG, 1T uE3, 2T total hCG, 2T uE3 and 2T AFP at risk 1:384.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 35 Age, 1T AFP, 1T free hCG, 1T uE3, 2T total hCG, 2T uE3 and 2T AFP at risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity
Goh 1996	29	1239	5	11066	0.85 [0.69, 0.95]	0.90 [0.89, 0.90]

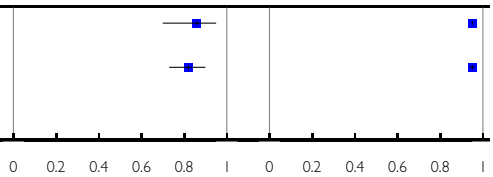


Test 36. Age, 1T NT, 2T total hCG and 2T AFP, 5FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 36 Age, 1T NT, 2T total hCG and 2T AFP, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Lam 2002	30	810	5	15392	0.86 [0.70, 0.95]	0.95 [0.95, 0.95]
Wald 2003b	70	51	15	974	0.82 [0.73, 0.90]	0.95 [0.94, 0.96]

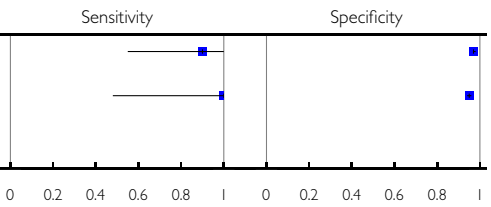


Test 37. Age, 1T NT, 2T total hCG and 2T AFP, risk 1:250.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 37 Age, 1T NT, 2T total hCG and 2T AFP, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity
Audibert 2001	9	97	1	3683	0.90 [0.55, 1.00]	0.97 [0.97, 0.98]
Benattar 1999	5	75	0	1576	1.00 [0.48, 1.00]	0.95 [0.94, 0.96]

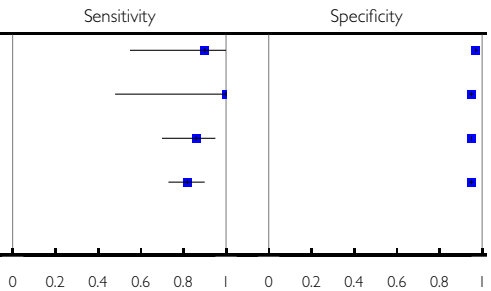


Test 38. Age, 1T NT, 2T total hCG and 2T AFP, mixture cutpoint.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 38 Age, 1T NT, 2T total hCG and 2T AFP, mixture cutpoint

Study	TP	FP	FN	TN	Sensitivity	Specificity
Audibert 2001	9	97	1	3683	0.90 [0.55, 1.00]	0.97 [0.97, 0.98]
Benattar 1999	5	75	0	1576	1.00 [0.48, 1.00]	0.95 [0.94, 0.96]
Lam 2002	30	810	5	15392	0.86 [0.70, 0.95]	0.95 [0.95, 0.95]
Wald 2003b	70	51	15	974	0.82 [0.73, 0.90]	0.95 [0.94, 0.96]

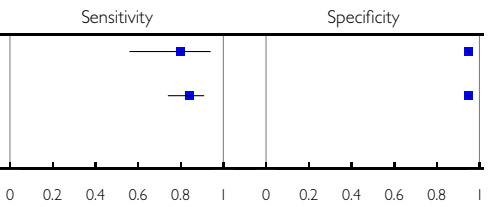


Test 39. Age, 1T NT, 2T free ßhCG and 2T AFP, 5FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 39 Age, 1T NT, 2T free hCG and 2T AFP, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Rozenberg 2002	16	274	4	5212	0.80 [0.56, 0.94]	0.95 [0.94, 0.96]
Wald 2003b	71	51	14	974	0.84 [0.74, 0.91]	0.95 [0.94, 0.96]

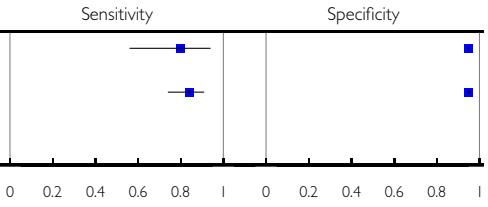


Test 40. Age, 1T NT, 2T free ßhCG and 2T AFP, mixture cutpoint.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 40 Age, 1T NT, 2T free hCG and 2T AFP, mixture cutpoint

Study	TP	FP	FN	TN	Sensitivity	Specificity
Rozenberg 2002	16	291	4	5195	0.80 [0.56, 0.94]	0.95 [0.94, 0.95]
Wald 2003b	71	51	14	974	0.84 [0.74, 0.91]	0.95 [0.94, 0.96]



Test 41. Age, 1T NT, 2T free ßhCG, 2T uE3 and 2T AFP, 5FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 41 Age, 1T NT, 2T free hCG, 2T uE3 and 2T AFP, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003b	75	51	10	974	0.88 [0.79, 0.94]	0.95 [0.94, 0.96]		

Test 42. Age, 1T NT, 2T total hCG, 2T uE3 and 2T AFP, 5FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 42 Age, 1T NT, 2T total hCG, 2T uE3 and 2T AFP, 5FPR

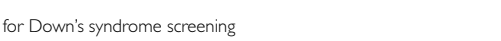
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003b	74	51	11	974	0.87 [0.78, 0.93]	0.95 [0.94, 0.96]		

Test 43. Age, 1T NT, 2T total hCG, 2T uE3 and 2T AFP, risk 1:250.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 43 Age, 1T NT, 2T total hCG, 2T uE3 and 2T AFP, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Babbur 2005	6	73	3	2643	0.67 [0.30, 0.93]	0.97 [0.97, 0.98]		
Herman 2002	17	12	6	496	0.74 [0.52, 0.90]	0.98 [0.96, 0.99]		

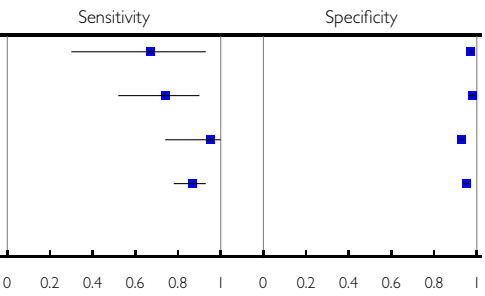


Test 44. Age, 1T NT, 2T total hCG, 2T uE3 and 2T AFP, mixture cutpoint.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 44 Age, 1T NT, 2T total hCG, 2T uE3 and 2T AFP, mixture cutpoint

Study	TP	FP	FN	TN	Sensitivity	Specificity
Babbur 2005	6	73	3	2643	0.67 [0.30, 0.93]	0.97 [0.97, 0.98]
Herman 2002	17	12	6	496	0.74 [0.52, 0.90]	0.98 [0.96, 0.99]
Schuchter 2001	18	652	1	8671	0.95 [0.74, 1.00]	0.93 [0.92, 0.94]
Wald 2003b	74	51	11	974	0.87 [0.78, 0.93]	0.95 [0.94, 0.96]

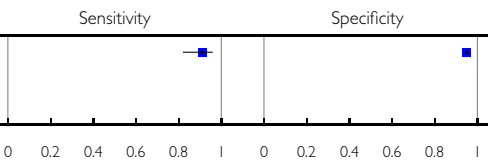


Test 45. Age, 1T NT, 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A, 5FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 45 Age, 1T NT, 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003b	77	51	8	974	0.91 [0.82, 0.96]	0.95 [0.94, 0.96]



Test 46. Age, 1T NT, 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A, 5FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 46 Age, 1T NT, 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003b	77	51	8	974	0.91 [0.82, 0.96]	0.95 [0.94, 0.96]		

Test 47. Age, 1T NT, 2T free ßhCG, 2T uE3, 2T AFP and 1T PAPP-A , 5FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 47 Age, 1T NT, 2T free hCG, 2T uE3, 2T AFP and 1T PAPP-A , 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003b	78	51	7	974	0.92 [0.84, 0.97]	0.95 [0.94, 0.96]		

Test 48. Age, 1T NT, 2T free ßhCG, 2T uE3, 2T AFP and 1T PAPP-A , risk 1:250.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 48 Age, 1T NT, 2T free hCG, 2T uE3, 2T AFP and 1T PAPP-A , risk 1:250

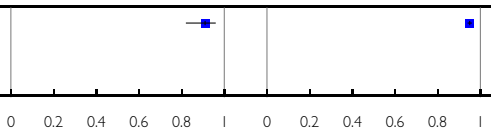
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003b	58	11	7	314	0.89 [0.79, 0.96]	0.97 [0.94, 0.98]		

Test 49. Age, 1T NT, 1T PAPP-A , 2T total hCG and 2T AFP, 5FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 49 Age, 1T NT, 1T PAPP-A , 2T total hCG and 2T AFP, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003b	77	51	8	974	0.91 [0.82, 0.96]	0.95 [0.94, 0.96]		

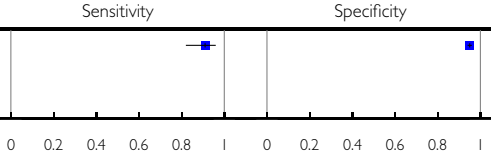


Test 50. Age, 1T NT, 1T PAPP-A , 2T free ßhCG and 2T AFP, 5FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 50 Age, 1T NT, 1T PAPP-A , 2T free hCG and 2T AFP, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003b	77	51	8	974	0.91 [0.82, 0.96]	0.95 [0.94, 0.96]		

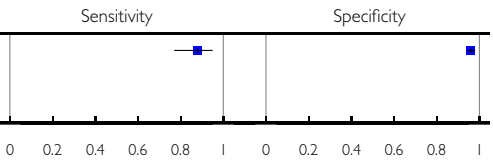


Test 51. Age, 1T NT, 1T PAPP-A , 2T free ßhCG and 2T AFP,risk 1:250.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 51 Age, 1T NT, 1T PAPP-A , 2T free hCG and 2T AFP,risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003b	57	12	8	313	0.88 [0.77, 0.95]	0.96 [0.94, 0.98]

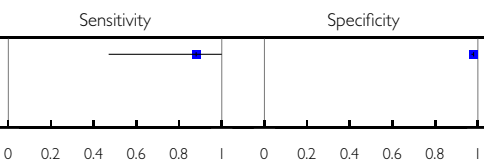


Test 52. Age, 1T NT, 1T PAPP-A , 2T free ßhCG and 2T AFP, risk 1:300.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 52 Age, 1T NT, 1T PAPP-A , 2T free hCG and 2T AFP, risk 1:300

Study	TP	FP	FN	TN	Sensitivity	Specificity
Rodrigues 2009	7	57	1	2225	0.88 [0.47, 1.00]	0.98 [0.97, 0.98]

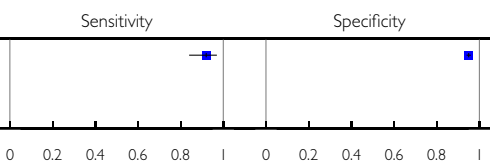


Test 53. Age, 1T NT, 1T PAPP-A, 2T total hCG, 2T uE3 and 2T AFP 5FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 53 Age, 1T NT, 1T PAPP-A, 2T total hCG, 2T uE3 and 2T AFP 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003b	78	51	7	974	0.92 [0.84, 0.97]	0.95 [0.94, 0.96]

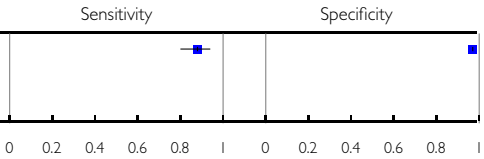


Test 54. Age, 1T NT, 1T PAPP-A , 2T total hCG, 2T uE3 and 2T AFP, risk 1:200.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 54 Age, 1T NT, 1T PAPP-A , 2T total hCG, 2T uE3 and 2T AFP, risk 1:200

Study	TP	FP	FN	TN	Sensitivity	Specificity
Okun 2008 Integrated	76	992	10	31149	0.88 [0.80, 0.94]	0.97 [0.97, 0.97]

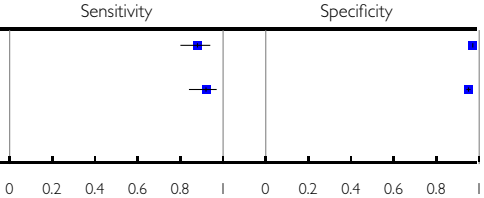


Test 55. Age, 1T NT, 1T PAPP-A, 2T total hCG, 2T uE3 and 2T AFP, mixed cutpoints.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 55 Age, 1T NT, 1T PAPP-A, 2T total hCG, 2T uE3 and 2T AFP, mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Okun 2008 Integrated	76	992	10	31149	0.88 [0.80, 0.94]	0.97 [0.97, 0.97]
Wald 2003b	78	51	7	974	0.92 [0.84, 0.97]	0.95 [0.94, 0.96]



Test 56. Age, 1T NT, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A, 5FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 56 Age, 1T NT, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Malone 2005	83	1677	4	31869	0.95 [0.89, 0.99]	0.95 [0.95, 0.95]		
Wald 2003b	79	51	6	974	0.93 [0.85, 0.97]	0.95 [0.94, 0.96]		

Test 57. Age, 1T NT, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A, risk 1:150.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 57 Age, 1T NT, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A, risk 1:150

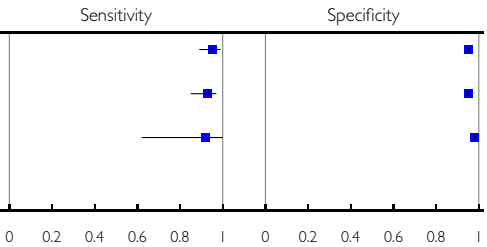
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2009	11	80	1	4835	0.92 [0.62, 1.00]	0.98 [0.98, 0.99]		

Test 58. Age, 1T NT, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A, mixed cutpoints.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 58 Age, 1T NT, 1T PAPP-A , 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A, mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Malone 2005	83	1677	4	31869	0.95 [0.89, 0.99]	0.95 [0.95, 0.95]
Wald 2003b	79	51	6	974	0.93 [0.85, 0.97]	0.95 [0.94, 0.96]
Wald 2009	11	80	1	4835	0.92 [0.62, 1.00]	0.98 [0.98, 0.99]

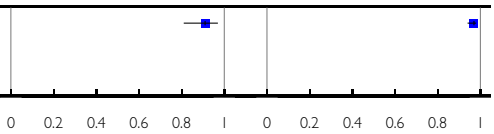


Test 59. Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:300.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 59 Age, 1T NT, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:300

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003b	59	11	6	314	0.91 [0.81, 0.97]	0.97 [0.94, 0.98]

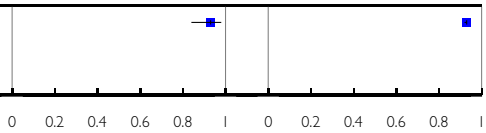


Test 60. Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A, 1:270.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 60 Age, 1T NT, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A, 1:270

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Aagaard-Tillery 2009	55	553	4	7230	0.93 [0.84, 0.98]	0.93 [0.92, 0.93]		

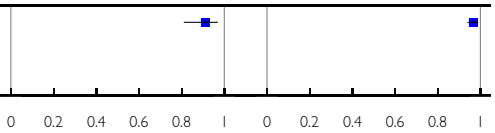


Test 61. Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:250.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 61 Age, 1T NT, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003b	59	10	6	315	0.91 [0.81, 0.97]	0.97 [0.94, 0.99]		

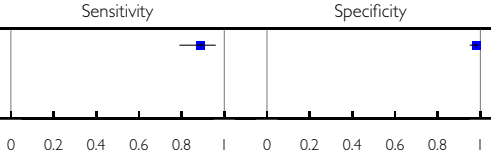


Test 62. Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:200.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 62 Age, 1T NT, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:200

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003b	58	8	7	317	0.89 [0.79, 0.96]	0.98 [0.95, 0.99]		

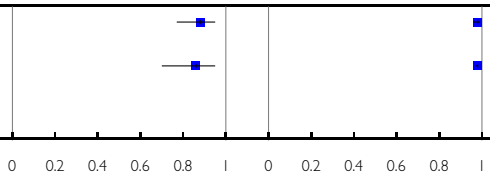


Test 63. Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:150.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 63 Age, 1T NT, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:150

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003b	57	6	8	319	0.88 [0.77, 0.95]	0.98 [0.96, 0.99]		
Wald 2009	30	219	5	9115	0.86 [0.70, 0.95]	0.98 [0.97, 0.98]		

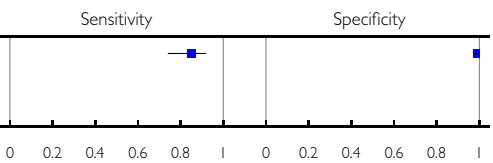


Test 64. Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:100.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 64 Age, 1T NT, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:100

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003b	55	4	10	321	0.85 [0.74, 0.92]	0.99 [0.97, 1.00]		

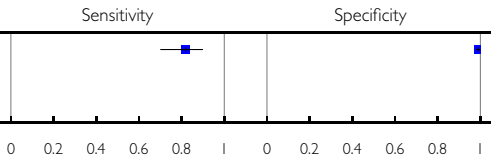


Test 65. Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:50.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 65 Age, 1T NT, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A,risk 1:50

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003b	53	2	12	323	0.82 [0.70, 0.90]	0.99 [0.98, 1.00]

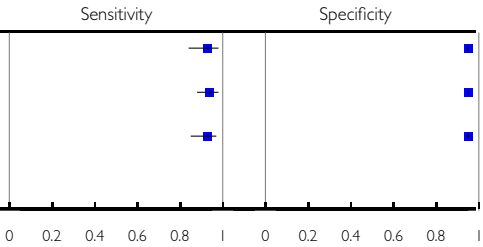


Test 66. Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A, 5FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 66 Age, 1T NT, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Aagaard-Tillery 2009	55	389	4	7394	0.93 [0.84, 0.98]	0.95 [0.94, 0.95]
Bestwick 2010	100	1132	6	21508	0.94 [0.88, 0.98]	0.95 [0.95, 0.95]
Wald 2003b	79	51	6	974	0.93 [0.85, 0.97]	0.95 [0.94, 0.96]



Test 67. Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A, 3FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 67 Age, 1T NT, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A, 3FPR

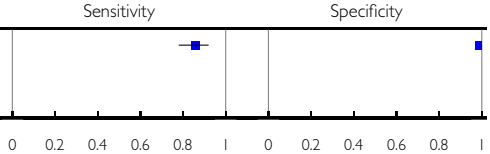
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Bestwick 2010	98	679	8	21961	0.92 [0.86, 0.97]	0.97 [0.97, 0.97]		

Test 68. Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A, 1FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 68 Age, 1T NT, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A, 1FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Bestwick 2010	91	226	15	22414	0.86 [0.78, 0.92]	0.99 [0.99, 0.99]		

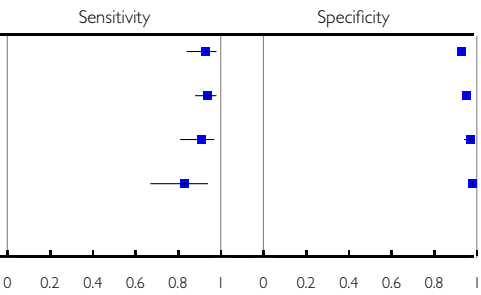


Test 69. Age, 1T NT, 1T PAPP-A , 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A, mixed cutpoints.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 69 Age, 1T NT, 1T PAPP-A , 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A, mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Aagaard-Tillery 2009	55	553	4	7230	0.93 [0.84, 0.98]	0.93 [0.92, 0.93]
Bestwick 2010	100	1132	6	21508	0.94 [0.88, 0.98]	0.95 [0.95, 0.95]
Wald 2003b	59	10	6	315	0.91 [0.81, 0.97]	0.97 [0.94, 0.99]
Wald 2009	30	219	6	9115	0.83 [0.67, 0.94]	0.98 [0.97, 0.98]

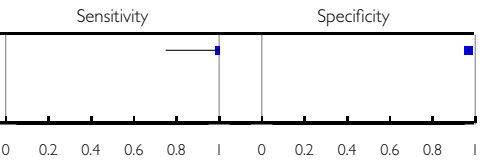


Test 70. Age, 1T NT, 1T PAPP-A, 1T free ßhCG, 2T total hCG, 2T uE3 and 2T AFP, risk 1:250.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 70 Age, 1T NT, 1T PAPP-A, 1T free hCG, 2T total hCG, 2T uE3 and 2T AFP, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity
Guanciali-Franchi 2010	13	170	0	4877	1.00 [0.75, 1.00]	0.97 [0.96, 0.97]



Test 71. Age, 1T NT, 1T PAPP-A, 1T free ßhCG, 2T uE3, 2T AFP, 2T total hCG and 2T Inhibin A, risk 1:150.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 71 Age, 1T NT, 1T PAPP-A, 1T free hCG, 2T uE3, 2T AFP, 2T total hCG and 2T Inhibin A, risk 1:150

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Malone 2005	82	3680	5	29779	0.94 [0.87, 0.98]	0.89 [0.89, 0.89]		

Test 72. ADAM 12 2T TO 1T RATIO.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 72 ADAM 12 2T TO 1T RATIO

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Baviera 2010	9	28	8	534	0.53 [0.28, 0.77]	0.95 [0.93, 0.97]		

Test 73. Stepwise: Age, 1T NT, 1T PAPP-A , 1T free ßhCG, if risk <1/30, 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A risk 1:270.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 73 Stepwise: Age, 1T NT, 1T PAPP-A , 1T free hCG, if risk <1/30, 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A risk 1:270

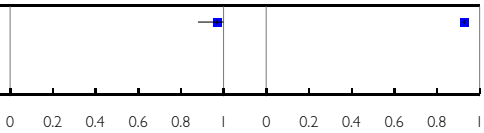
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Cuckle 2008	79	1632	7	30637	0.92 [0.84, 0.97]	0.95 [0.95, 0.95]		

Test 74. Stepwise: Age, 1T NT, 1T PAPP-A , 1T free ßhCG, if risk <1/30, 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A risk 1:270.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 74 Stepwise: Age, 1T NT, 1T PAPP-A , 1T free hCG, if risk <1/30, 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A risk 1:270

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Aagaard-Tillery 2009	57	560	2	7223	0.97 [0.88, 1.00]	0.93 [0.92, 0.93]		

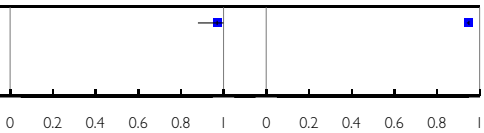


Test 75. Stepwise: Age, 1T NT, 1T PAPP-A , 1T free ßhCG, if risk <1/30, 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A 5% FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 75 Stepwise: Age, 1T NT, 1T PAPP-A , 1T free hCG, if risk <1/30, 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Aagaard-Tillery 2009	57	389	2	7394	0.97 [0.88, 1.00]	0.95 [0.94, 0.95]		



Test 76. Stepwise: Age, 1T NT, 1T PAPP-A , if risk <1:100, 2T free ßhCG, 2T uE3, 2T AFP, risk 1:250.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 76 Stepwise: Age, 1T NT, 1T PAPP-A , if risk <1:100, 2T free hCG, 2T uE3, 2T AFP, risk 1:250

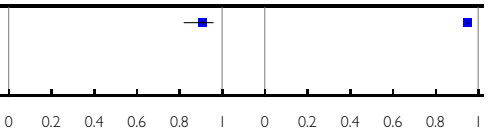
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Habayeb 2010	11	48	1	1447	0.92 [0.62, 1.00]	0.97 [0.96, 0.98]		

Test 77. Contingent: Age, 1T NT, 1T PAPP-A , 1T free ßhCG, if risk 1/30-1/1500, 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A risk 1:270.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 77 Contingent: Age, 1T NT, 1T PAPP-A , 1T free hCG, if risk 1/30-1/1500, 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A risk 1:270

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Cuckle 2008	78	1467	8	30802	0.91 [0.82, 0.96]	0.95 [0.95, 0.96]		

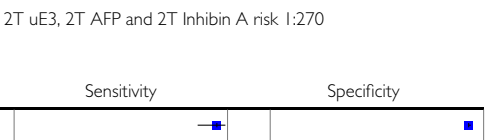


Test 78. Contingent: Age, 1T NT, 1T PAPP-A , 1T free ßhCG, if risk 1/30-1/1500, 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A risk 1:270.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 78 Contingent: Age, 1T NT, 1T PAPP-A , 1T free hCG, if risk 1/30-1/1500, 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A risk 1:270

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Aagaard-Tillery 2009	56	514	3	7269	0.95 [0.86, 0.99]	0.93 [0.93, 0.94]		

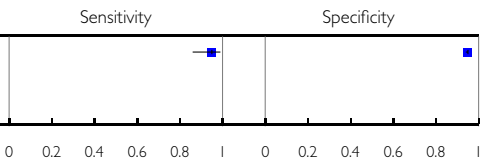


Test 79. Contingent: Age, 1T NT, 1T PAPP-A , 1T free ßhCG, if risk 1/30-1/1500, 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A 5%FPR.

Review: First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening

Test: 79 Contingent: Age, 1T NT, 1T PAPP-A , 1T free hCG, if risk 1/30-1/1500, 2T free hCG, 2T uE3, 2T AFP and 2T Inhibin A 5%FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Aagaard-Tillery 2009	56	389	3	7394	0.95 [0.86, 0.99]	0.95 [0.94, 0.95]



ADDITIONAL TABLES

Table 1. Direct comparisons of the diagnostic accuracy of the six most evaluated test strategies

Ratio of DORs (95% CI); P value (Studies)	1T PAPP-A, 2T total hCG, 2T uE3 and 2T AFP	1T PAPP-A, 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A	1T NT, 2T total hCG and 2T AFP	1T NT, 2T total hCG, 2T uE3 and 2T AFP	1T NT, 1T PAPP-A, 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A
1T PAPP-A, 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A	1.43 (0.39, 5.25); P = 0.49 (*K* = 1)				
1T NT, 2T total hCG and 2T AFP	0.86 (0.25, 2.96); P = 0.75 (*K* = 1)	0.60 (0.16, 2.22); P = 0.34 (*K* = 1)			
1T NT, 2T total hCG, 2T uE3 and 2T AFP	1.23 (0.33, 4.57); P = 0.68 (*K* = 1)	0.86 (0.22, 3.43); P = 0.78 (*K* = 1)	1.44 (0.38, 5.41); P = 0.49 (*K* = 1)		
1T NT, 1T PAPP-A, 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A	2.97 (0.53, 16.6); P = 0.15 (*K* = 1)	2.08 (0.35, 12.3); P = 0.32 (*K* = 1)	3.48 (0.62,19.6); P = 0.12 (*K* = 1)	2.41 (41, 14.3); P = 0.24 (*K* = 1)	

Table 1. Direct comparisons of the diagnostic accuracy of the six most evaluated test strategies (*Continued*)

1T NT, 1T PAPP-A, 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A	2.41 (0.53, 11.0); P = 0.18 (*K* = 1)	1.69 (0.35, 8.16); P = 0.41 (*K* = 2)	2.82 (0.61, 13.0); P = 0.13 (*K* = 1)	1.96 (0.40, 9.53); P = 0.30 (*K* = 1)	1.87 (0.57, 6.06); P = 0.26 (*K* = 2)

Direct comparisons were made using only data from studies that compared each pair of tests in the same population. Ratio of diagnostic odds ratios (DORs) were computed by division of the DOR for the test in the row by the DOR for the test in the column. If the ratio of DORs is greater than one, then the diagnostic accuracy of the test in the row is higher than that of the test in the column; if the ratio is less than one, the diagnostic accuracy of the test in the column is higher than that of the test in the row. All test combinations include maternal age. All test comparisons that were evaluated by only one study were from Wald 2003b.

1T = first trimester; **2T** = second trimester; **K** = number of studies; **CI** = confidence interval

AFP = alpha-fetoprotein; **ßhCG** = beta human chorionic gonadotrophin; **FPR** = false positive rate; **hCG** = human chorionic gonadotrophin; **NT** = nuchal translucency; **PAPP-A** = pregnancy-associated plasma protein-A; **uE3** = unconjugated oestriol.

Table 2. Indirect comparisons of the diagnostic accuracy of the six most evaluated test strategies

Ratio of DORs (95% CI); P value		**1T PAPP-A, 2T total hCG, 2T uE3 and 2T AFP**	**1T PAPP-A, 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A**	**1T NT, 2T total hCG and 2T AFP**	**1T NT, 2T total hCG, 2T uE3 and 2T AFP**	**1T NT, 1T PAPP-A, 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A**
	DOR (95% CI) Studies	96 (48, 190) *K* =4	114 (62, 210) *K* = 3	103 (49, 215) *K* = 4	109 (51, 233) *K* = 4	214 (125, 367) *K* = 4
1T PAPP-A, 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A	114 (62, 210) *K* = 3	1.19 (0.61, 2.32) ; P = 0.58				
1T NT, 2T total hCG and 2T AFP	103 (49, 215) *K* = 4	1.08 (0.51, 2.36) ; P = 0.83	0.91 (0.43, 1.90) ; P = 0.78			
1T NT, 2T total hCG, 2T uE3 and 2T AFP	109 (51, 233) *K* = 4	1.14 (0.54, 2.42) ; P = 0.71	0.96 (0.45, 2.03) ; P = 0.90	1.06 (0.47, 2.41) ; P = 0.88		
1T NT, 1T PAPP-A, 2T free ßhCG, 2T uE3, 2T AFP and 2T Inhibin A	214 (125, 367) *K* = 4	2.24 (1.00, 5.00) ; P = 0.049	1.88 (0.88, 3.99) ; P = 0.094	2.08 (0.89, 4.87) ; P = 0.09	1.96 (0.82, 4.67) ; P = 0.12	

Table 2. Indirect comparisons of the diagnostic accuracy of the six most evaluated test strategies (*Continued*)

1T NT, 1T PAPP-A, 2T total hCG, 2T uE3, 2T AFP and 2T Inhibin A	339 (163, 705) *K* = 3	3.55 (1.28, 9.89) ; P = 0.019	2.98 (1.14; 7.80) ; P = 0.029	3.29 (1.15, 9.47) ; P = 0.030	3.11 (1.07, 9.07) ; P = 0.039	1.58 (0.64, 3.95) ; P = 0.30

Indirect comparisons were made using all available data. Ratio of diagnostic odds ratios (DORs) were computed by division of the DOR for the test in the row by the DOR for the test in the column. If the ratio of DORs is greater than one, then the diagnostic accuracy of the test in the row is higher than that of the test in the column; if the ratio is less than one, the diagnostic accuracy of the test in the column is higher than that of the test in the row. All test combinations include maternal age.

1T = first trimester; **2T** = second trimester; **K** = number of studies; **CI** - confidence interval.

AFP = alpha-fetoprotein; **ßhCG** = beta human chorionic gonadotrophin; **FPR** = false positive rate; **hCG** = human chorionic gonadotrophin; **NT** = nuchal translucency; **PAPP-A** = pregnancy-associated plasma protein-A; **uE3** = unconjugated oestriol.

Table 3. Comparison of integrated, contingent and stepwise strategy for a septuple combination of serum tests and first trimester nuchal translucency

Test combination	Screening policy	Study	Women (cases)	Sensitivity (95% CI)	Specificity (95% CI)	Threshold
First trimester NT, PAPP-A and free ßhCG, and second trimester uE3, AFP, total hCG and inhibin A	Integrated	Malone 2005	33,546 (87)	94 (87, 98)	89 (89, 89)	1:150 risk
First trimester NT, PAPP-A and free ßhCG, if risk <1:30 invasive testing is offered, if risk 1:30-1:1500, second trimester total hCG, uE3, AFP and inhibin A is performed	Contingent	Cuckle 2008	32,355 (86)	91 (82, 96)	95 (95, 96)	1:270 risk
First trimester NT, PAPP-A and free ßhCG, if risk <1:30 invasive testing is offered, if ≥ 1:30 second trimester	Stepwise	Cuckle 2008	32,355 (86)	92 (84, 97)	95 (95, 95)	1:270 risk

Table 3. Comparison of integrated, contingent and stepwise strategy for a septuple combination of serum tests and first trimester nuchal translucency (*Continued*)

total hCG, uE3, AFP and inhibin A is performed						

AFP = alpha-fetoprotein; **ßhCG** = beta human chorionic gonadotrophin; **FPR** = false positive rate; **hCG** = human chorionic gonadotrophin; **NT** = nuchal translucency; **PAPP-A** = pregnancy-associated plasma protein-A; **uE3** = unconjugated oestriol.
CI - confidence interval.

Table 4. Maternal age, reference standard and study design characteristics of included studies

Study	Maternal age (years)*	Reference standard†	Withdrawals explained?	Study design
Aagaard-Tillery 2009	30.6 (SD 6.1)	Karyotyping or follow-up to birth	Of 33,546 trial participants only 7842 women with complete information for all screening tests and genetic sonography were included in the study	Prospective cohort
Audibert 2001	30.1, all < 38, 86% < 35, 14% ≥35	Prenatal karyotype conducted (in 7. 6% of patients) depending on presence of risk >1/125, high maternal age, parental anxiety, history of chromosomal defects or parental translocation or abnormal second trimester scan. Cytogenetic testing of newborns with suspected abnormali- ties. Postmortum on terminations of pregnancy or miscarriages. Follow-up to neonatal examination in newborns	35 women were lost to follow-up (they had all had normal NT results). 340 women who did not want second trimester serum screening withdrew from that part of the study. Women lost to follow-up were excluded in the final analysis. All detected cases were terminated	Prospective consecutive series
Babbur 2005	Median 37 (range 19 to 46)	Invasive testing offered to women with NT > 3 mm or risk > 1:250 as defined by combined NT and serum results CVS from 11 weeks, amniocentesis from 15 weeks) . Rapid in situ hybridisa-	463 patients having NT did not go on to have second trimester serum testing. Women with miscarriages excluded	Prospective cohort

Table 4. Maternal age, reference standard and study design characteristics of included studies (*Continued*)

		tion test in patients with risk > 1:30. No details given of any follow-up to birth		
Baviera 2010	35.3 for Down's cases, 30.4 for controls	Amniocentesis or follow-up to birth	No details of withdrawals given.	Case control
Benattar 1999	32 (16 to 46), 8.3% > 35	Amniocentesis due to maternal age > 38 years (6.1% or women). Karyotyping encouraged for women with positive result on one or more index test. No details of reference standard for index test negative women	No details of withdrawals given. 12 patients were lost to follow-up due to miscarriages	Prospective cohort
Bestwick 2010	Median 39 for Down's cases, 34 for non-Down's cases	Karyotyping or follow-up to birth	No details of withdrawals given.	Retrospective cohort
Cuckle 2008	Not reported	Karyotyping or follow-up to birth	No details of withdrawals given.	Prospective cohort
Goh 1996	33	Karyotyping or follow-up to birth	No details of withdrawals given.	Cohort
Guanciali-Franchi 2010	31.8	Karyotyping or follow-up to birth	No details of withdrawals given.	Prospective cohort
Habayeb 2010	Median 35.4 (range 18 to 49)	Karyotyping or follow-up to birth	No details of withdrawals given.	Cohort
Herman 2002	Not reported	Karyotyping or follow-up to birth	No details of withdrawals given.	Case control
Lam 2002	30.5 (19% ≥35) (unaffected pregnancies)	Women considered high risk offered CVS (0.7%) or amniocentesis (11.8%). Follow-up to birth	Details given for patients excluded and those without follow-up data	Prospective cohort
Malone 2005	21.6% aged 35 and above	Amniocentesis (offered to women with positive results from any screening test) or follow-up to birth	Details given for patients who did not undergo different index tests. Unclear which patients did not have follow-up data. Appears that aborted/miscarried foetuses did	Prospective cohort

Table 4. Maternal age, reference standard and study design characteristics of included studies (*Continued*)

			not have follow-up	
Okun 2008 Integrated	32	Karyotyping or follow-up to birth	2614 (8%) of women undergoing integrated screening did not return for the second trimester part of the test	Prospective cohort
Palomaki 2006	33.9 (SD 4.4) for Down's cases, 35.9 (SD 3.6) for controls	Karyotyping or follow-up to birth	No details of withdrawals given.	Case control
Rodrigues 2009	30. 6 for integrated screening, 30.9 for serum integrated screening	Karyotyping or follow-up to birth	No details of withdrawals given.	Retrospective cohort
Rozenberg 2002	30.5 (18 to 37)	Amniocentesis offered to patients with NT > 3 mm or serum marker risk was > 1:250. Follow-up to birth	No details of withdrawals given. 3.4% of patients were lost to follow-up and were excluded from the study. This included 113 women (1.2%) with miscarriages	Prospective cohort
Schuchter 2001	28 (range 15 to 46), 10.7% aged 35 and above	CVS (offered to patients with first trimester NT > 3.5 mm), amniocentesis (offered to patients with first trimester NT 2.5 to 3.4, high risk on second trimester serum testing (> 1:250) and those > 35 years) or follow-up to birth	No details of withdrawals given. Women having miscarriages were excluded from the study	Retrospective cohort
Wald 2003b	Not reported	Invasive testing (following second trimester screening) or follow-up to birth	No details of withdrawals given.	Case control
Wald 2009	Median 33 (range 15 to 51), 20% aged 37 and above	Karyotyping or follow-up to birth	No details of withdrawals given.	Retrospective cohort
Wright 2010 FASTER trial	Not reported	Karyotyping or follow-up to birth	No details of withdrawals given.	Case control

Table 4. Maternal age, reference standard and study design characteristics of included studies (*Continued*)

Wright 2010 North York	Not reported	Karyotyping or follow-up to birth	No details of withdrawals given.	Case control

CVS = chorionic villus sampling; **NT** = nuchal translucency; **SD** = standard deviation
*Mean maternal age presented unless otherwise indicated.
†In all studies the choice of reference standard was dependent on the results of the index test.

APPENDICES

Appendix 1. Search Strategy

Database: Ovid MEDLINE

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1 exp Prenatal Diagnosis/
2 nuchal translucency.mp.
3 exp Pregnancy-Associated Plasma Protein-A/
4 pregnancy associated plasma protein a.mp.
5 papp-a.mp.
6 exp Chorionic Gonadotropin, beta Subunit, Human/
7 (b-hcg or bhcg).mp.
8 human chorionic gonadotropin.mp.
9 exp alpha-Fetoproteins/
10 alphafetoprotein$.mp.
11 alpha-fetoprotein$.mp.
12 afp.mp.
13 (unconjugated estriol or unconjugated oestriol).mp.
14 ue3.mp.
15 exp INHIBINS/
16 inhibin a.mp.
17 ultrasound.mp.
18 amniocentesis/
19 chorion$ vill$ sampling.mp.
20 Chorionic Villi-Sampling/
21 nasal bone.mp.
22 tricuspid regurgitation.mp.
23 ductus venosus.mp
24 marker$.mp.
25 screen$.mp.
26 detect$.mp.
27 accura$.mp.
28 predict$.mp.
29 ROC.mp.
30 ROC curve/
31 AUC.mp.

32 Area under curve/
33 exp false negative reactions/ or exp false positive reactions/
34 (false positive$ or false negative$).mp.
35 likelihood ratio$.mp.
36 sensitiv$.mp.
37 specific$.mp.
38 diagnos$.ti,ab.
39 "reproducibility of results".mp.
40 reference value$.mp.
41 reference standard$.mp.
42 exp Down Syndrome/
43 downs syndrome.mp.
44 down syndrome.mp.
45 trisomy 21.mp.
46 Aneuploidy/
47 aneuploidy.mp.
48 Mosaicism/
49 mosaicism.mp.
50 or/1-41
51 or/42-49
52 50 and 51
53 (antenatal$ or prenatal$ or trimester$ or pregnan$ or fetus or foetus or fetal or foetal).mp.
54 52 and 53
55 animal/ not (humans/ and animal/)
56 54 not 55
****

EMBASE via Dialog Datastar

1. PRENATAL-DIAGNOSIS#.DE.
2. FETUS-ECHOGRAPHY#.DE.
3. PREGNANCY-ASSOCIATED-PLASMA-PROTEIN-A#.DE.
4. CHORIONIC-GONADOTROPIN-BETA-SUBUNIT#.DE.
5. HCG.AB.
6. PAPP.AB.
7. ALPHA-FETOPROTEIN#.DE.
8. AFP.AB.
9. ALPHA ADJ FETOPROTEIN$
10. ALPHAFETOPROTEIN$
11. BETA ADJ HUMAN ADJ CHORIONIC ADJ GONADOTROPIN
12. PREGNANCY ADJ ASSOCIATED ADJ PLASMA ADJ PROTEIN
13. (UNCONJUGATED ADJ ESTRIOL OR UNCONJUGATED ADJ OESTRIOL).TI.
14. (UNCONJUGATED ADJ ESTRIOL OR UNCONJUGATED ADJ OESTRIOL).AB.
15. UE3
16. INHIBIN-A#.DE.
17. INHIBIN ADJ A
18. ULTRASOUND
19. AMNIOCENTESIS
20. CHORION-VILLUS-SAMPLING.DE.
21. NASAL ADJ BONE
22. TRICUSPID ADJ REGURGITATION
23. DUCTUS ADJ VENOSUS
24. MARKER OR MARKERS
25. SCREEN OR SCREENING

26. DETECT OR DETECTING OR DETECTION
27. FALSE ADJ POSITIVE$
28. FALSE ADJ NEGATIVE$
29. SENSITIVITY OR SENSITIVE OR SENSITIVITIES
30. SPECIFICITY OR SPECIFICITIES
31. (DIAGNOSE OR DIAGNOSIS OR DIAGNOSTIC OR DIAGNOSTICS OR DIAGNOSES OR DIAGNOSED).TI.
32. (DIAGNOSE OR DIAGNOSIS OR DIAGNOSTIC OR DIAGNOSTICS OR DIAGNOSES OR DIAGNOSED).AB.
33. ROC.AB.
34. AUC.AB.
35. AREA-UNDER-THE-CURVE.DE.
36. ROC-CURVE.DE.
37. ACCURA$
38. PREDICT$
39. REPRODUCIBILITY.DE.
40. REFERENCE ADJ VALUE$
41. REFERENCE-VALUE.DE.
42. REFERENCE ADJ STANDARD$
43. DOWN-SYNDROME#.DE.
44. DOWN ADJ SYNDROME OR DOWNS ADJ SYNDROME
45. TRISOMY ADJ '21'
46. MOSAICISM
47. ANEUPLOIDY
48. ANTENATAL$ OR PRENATAL$ OR PREGNANCY OR PREGNANT OR TRIMESTER$ OR MATERNAL OR FETUS OR FOETUS OR FOETAL OR FETAL
49. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23 OR 24 OR 25 OR 26 OR 27 OR 28 OR 29 OR 30 OR 31 OR 32 OR 33 OR 34 OR 35 OR 36 OR 37 OR 38 OR 39 OR 40 OR 42
50. 43 OR 44 OR 45 OR 46 OR 47
51. 48 AND 49 AND 50
52. HUMAN=YES
53. 51 AND 52

ADJ = adjacent AB = abstract
TI = title $ = truncation symbol DE = descriptor (similar to MeSH)
****

CINAHL via OVID
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1 exp Prenatal Diagnosis/
2 nuchal translucency.mp.
3 pregnancy associated plasma protein.mp.
4 papp$.ti,ab.
5 exp Gonadotropins, chorionic/
6 (b-hcg or bhcg).mp.
7 human chorionic gonadotropin.mp.
8 exp alpha-Fetoproteins/
9 alphafetoprotein$.mp.
10 alpha-fetoprotein$.mp.
11 afp.mp.
12 (unconjugated estriol or unconjugated oestriol).mp.
13 ue3.mp.
14 inhibin$.mp.

15 ultrasound.mp.
16 amniocentesis/
17 chorion$ vill$ sampling.mp.
18 Chorionic Villi-Sampling/
19 nasal bone.mp.
20 tricuspid regurgitation.mp.
21 ductus venosus.mp.
22 marker$.mp.
23 screen$.mp.
24 detect$.mp.
25 accura$.mp.
26 predict$.mp.
27 ROC.mp.
28 ROC curve/
29 AUC.mp.
30 "area under curve".mp.
31 exp false negative reactions/ or exp false positive reactions/
32 (false positive$ or false negative$).mp.
33 likelihood ratio$.mp.
34 sensitiv$.mp.
35 specific$.mp.
36 diagnos$.ti,ab.
37 "reproducibility of results".mp.
38 reference value$.mp.
39 reference standard$.mp.
40 exp Down Syndrome/
41 downs syndrome.mp.
42 down syndrome.mp.
43 trisomy 21.mp.
44 aneuploidy.mp.
45 mosaicism.mp.
46 (antenatal$ or prenatal$ or trimester$ or pregnan$ or fetus or foetus or fetal or foetal).mp.
47 or/1-39
48 or/40-45
49 47 and 48 and 46
****

Search terms and instructions for Biosis

The following search terms were entered separately in standard search box (select 'Titles/subject/abstract' from the drop-down box on the right of the search box).

1. "reference standard*"
2. "reference value*"
3. "reproducibility of results"
4. diagnos*
5. sensitiv*
6. specific*
7. "likelihood ratio*"
8. "false negative*
9. "false positive"
10. "area under curve"
11. ROC
12. AUC

13. predict*
14. detect*
15. marker*
16. screen*
17. accura*
18. "ductus venosus"
19. "nasal bone"
20. "tricuspid regurgitation"
21. "chorion* vill* sampling"
22. amniocentesis
23. ultrasound
24. inhibin*
25. "unconjugaed oestriol"
26. "unconjugated estriol"
27. afp
28. "alpha fetoprotein*"
29. alphafetoprotein*
30. " bhcg"
31. "human chorionic gonadotrophin"
32. "papp a"
33. "pregnancy associated plasma protein"
34. "nuchal translucency"
35. foetal
36. fetal
37. foetus
38. foetal
39. prenatal*
40. antenatal*
41. pregnan*
42. maternal*
43. "trisomy 21"
44. mosaicism
45. "down* syndrome"

The search then used the history function to combine terms:
1-34 - combine using OR
35 - 42 - combine using OR
43 - 45 - combine using OR
The three sets were combined using AND

The combined search strategy had the form
(((((((al: "trisomy 21") or (al: (mosaicism))) or (al: "down* syndrome"))) and ((((((((((((((((((((((((((((((((((al: "reference standard*") or (al: "reference value*")) or (al: "reproducibility of results")) or (al: (diagnos*))) or (al: (specific*))) or (al: (sensitiv*))) or (al: "likelihood ratio*")) or (al: "false negative*")) or (al: "false positive*")) or (al: "area under curve")) or (al: (auc))) or (al: (roc))) or (al: (predict*))) or (al: (accura*))) or (al: (detect*))) or (al: (screen*))) or (al: (marker*))) or (al: "ductus venosus")) or (al: "tricuspid regurgitation")) or (al: "nasal bone")) or (al: "chorion* vill* sampling")) or (al: (amniocentesis))) or (al: (ultrasound))) or (al: (inhibin*))) or (al: "unconjugated oestriol")) or (al: "unconjugated estriol")) or (al: (afp))) or (al: "alpha feto protein*")) or (al: "alpha fetoprotein*")) or (al: "b hcg")) or (al: "human chorionic gonadotropin")) or (al: "papp a")) or (al: "pregnancy associated plasma protein")) or (al: "nuchal translucency")))) and ((((((((al: (foetal)) or (al: (fetal))) or (al: (foetus))) or (al: (fetus))) or (al: (pregnan*))) or (al: (trimester*))) or (al: (prenatal*))) or (al: (antenatal*))))))

****

The Database of Abstracts of Reviews of Effectiveness (DARE), National Research Register and Health Services Research Projects in Progress database
:

1. Down syndrome (MeSH)
2. down* next syndrome
3. trisomy
4. aneuploidy
5. mosaicism
6. OR/ 1-5

****
MEDION (http://www.mediondatabase.nl/)

ICPC code for pregnancy - 'W'.
****
The Database of Systematic Reviews and Meta-Analyses in Laboratory Medicine - download the database to a .pdf file and search for the following terms separately:

Down
Trisomy
Aneuploidy
Pregnant
Pregnancy
Pregnancies
Mosaicism
****

Appendix 2. Glossary of terms (adapted in part from the UK National Screening Committee Glossary)

Abnormal ductus venosus flow velocity	The ductus venosus is a vessel in the fetus which allows oxygenated blood from the placenta to bypass the fetal liver and flow straight to the heart. In conditions such as Down's syndrome the pressure in this vessel can be abnormally high
Absent nasal bone	Absence of the bone that forms the bridge of the nose, which may be detected at ultrasound scan during early pregnancy
Affected individuals	Those individuals who are affected by the disorder for which they are being screened
Amniocentesis	Amniocentesis is an invasive procedure which involves taking a small sample of the amniotic fluid (liquor) surrounding the baby, using a needle which goes through the abdominal wall into the uterus, and is usually performed after 15 weeks' gestation
Chorionic villus sampling (CVS)	Chorionic villus sampling involves taking a sample of the placental tissue using a needle which goes through the abdominal wall and uterus or a cannula through the cervix. It is usually performed between 10 and 13 weeks' gestation
Combined test	First trimester test (up to 13 + 6 weeks of pregnancy) based on combining nuchal translucency measurement with free beta-hCG, pregnancy-associated plasma protein A (PAPP-A) and the woman's age

(*Continued*)

Diagnostic accuracy	The amount of agreement between the information from the index test and the reference standard (see below)
Diagnostic test	A definitive test, performed after a positive screening test result that gives a diagnosis (i.e. yes or no)
Double test	Second trimester test (from 13 + 6 up to 24 weeks of pregnancy) based on the measurement of alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG ß either free beta-hCG or total hCG), together with the woman's age
First trimester	Pregnancy from conception up to 13 weeks and 6 days.
Iatrogenic	A disease or condition in a patient occurring as a result of treatment
Index test	A test or group of tests being evaluated in a systematic review
Integrated test	Measurements performed at different times of pregnancy combined into a single test result. Unless otherwise specified, 'integrated test' refers to the combination of nuchal translucency measurement and PAPP-A in the first trimester, with the quadruple test (see below) in the second
Mosaicism	This is a condition in which person has some cells containing a normal number of chromosomes, and some containing an abnormal number. The more abnormal cells there are, the greater the effect
Multiple of the median (MOM)	The serum test concentration for a pregnant woman divided by the average (median) for unaffected pregnancies in a defined population at the same stage of pregnancy
Quadruple test	Second trimester test (from 13 + 6 up to 24 weeks of pregnancy) based on the measurement of AFP, uE3, free beta-hCG (or total hCG), and inhibin-A together with the woman's age
Reference Standard	The best available method for establishing the presence or absence of the target disease or condition
Second trimester	Pregnancy from 14 weeks to 28 weeks' gestation. Note that for the purposes of this Cochrane review, second trimester testing refers to the period of 14 to 24 weeks' gestation
Tricuspid regurgitation	Leakiness of or backflow of blood through the tricuspid valve of the heart. The tricuspid valve separates the upper and lower chambers of the right side of the heart
Triple test	Second trimester test (from 14 up to 24 weeks of pregnancy) based on the measurement of AFP, unconjugated oestriol (uE3), and hCG (either total hCG or free beta-hCG) together with the woman's age
Trisomy	The presence of an extra chromosome resulting in three copies of a particular chromosome instead of the normal two

(*Continued*)

Translocation	Part of one chromosome is broken off and attached to another chromosome. This does not usually cause the individual any problems as they have a normal amount of chromosomes, but in an abnormal arrangement. It can be passed on as an extra chromosome to offspring, resulting in conditions such as Down's syndrome

CONTRIBUTIONS OF AUTHORS

KA undertook the searches, applied eligibility criteria, extracted and entered data and wrote the first and second draft of the review.

ZA applied eligibility criteria, provided senior clinical input, oversaw the review process, and approved the final draft of the review.

JD supervised and planned the review, checked data extraction, supervised statistical analyses and wrote the second draft of the review.

JP applied eligibility criteria, provided senior clinical input, oversaw the review process, and approved the final draft of the review.

BG checked data extraction and undertook statistical analyses.

MP applied eligibility criteria, extracted and entered data for the updated literature search, and entered characteristics of studies information.

YT checked data extraction, undertook statistical analyses and wrote parts of the first draft of the review.

DECLARATIONS OF INTEREST

S Kate Alldred was supported by a project grant from the NIHR Health Technology Assessment Programme.

Boliang Guo: none known.

Jonathan J Deeks : none known.

Zarko Alfirevic (ZA) is Director of Harris Wellbeing Preterm Birth Centre which is grant funded by the charity Wellbeing of Women. This grant is administered by University of Liverpool and Zarko Alfirevic is not paid directly. He is the principal investigator or co-investigator on several grants from public funders including National Institute of Health Research, British Medical Association, European Commission and WHO. He has received research support in the past from Perkin Elmer and Alere for research related to pre-eclampsia and preterm birth prevention. These grants were administered by his employers and ZA did not benefit directly. ZA is also a Co-coordinating Editor of Cochrane Pregnancy and Childbirth.

James P Neilson received an award from the UK NIHR to facilitate a panel of Cochrane systematic reviews on Down's syndrome.

Mary Pennant: none known.

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DIFFERENCES BETWEEN PROTOCOL AND REVIEW

The protocol intended to investigate several additional outcomes downstream from test accuracy, should they be reported in the test accuracy studies. When we attempted to extract this information however, it was found to be available in very few studies, and where such information was found it was difficult to extract meaningful data to allow for comparison between studies, as data were not reported in a universal manner. In several studies such outcomes were estimated rather than measured. Often they were not reported at all. The outcomes stated in the protocol which have not been included are: harms of testing; need for further testing; side effects of test; interventions and side effects; other abnormalities detected by testing; spontaneous miscarriage; miscarriage subsequent to invasive procedure, with or without normal karyotype; fetal karyotype; termination of pregnancy (prior to definitive testing or in a karyotypically normal pregnancy and following confirmation of Down's syndrome or following detection of other chromosomal abnormalities); stillbirth; livebirth of affected and unaffected fetus; uptake of definitive testing by women.

The following refinements to the eligibility criteria were imposed to ensure that the quality of the included literature remained high. We excluded studies that identified fewer than five Down's syndrome pregnancies in their study population. We excluded studies that had less than 80% follow-up of participants.

In addition, the analytical strategy was informed by the volume of tests and studies included, and developed so that we focused on key tests and test combinations by a) only meta-analysed tests that were included in four or more studies or b) showed more than 70% sensitivity for more than 95% specificity. In addition, a requirement that a minimum of 10 studies for a single test was required before subgroup analysis was undertaken. Consequently several possible sources of heterogeneity were not investigated due to lack of data.

NOTES

This review belongs to a suite of reviews examining antenatal screening for Down's syndrome which includes:

- First trimester serum tests for Down's syndrome screening (Alldred 2015);
- Urine tests for Down's syndrome screening (Alldred 2015a)
- Second trimester serum tests for Down's syndrome screening (Alldred 2012);
- First trimester ultrasound tests alone or in combination with first trimester serum tests for Down's syndrome screening (in press)
- First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening (this review).

The plans for these reviews were described in a generic protocol (Alldred 2010) published in the Cochrane Library in 2010. The project as a whole has been much larger than initially anticipated, both in terms of size and statistical complexity. The initial search was

completed in 2007 and an updated search in August 2011. After identifying studies appropriate for inclusion, a significant amount of time has been devoted to data management and analysis.

The authors are conscious of the time lag from the latest literature search to publication, and the potential for the introduction of new urine tests in this time frame. The authors are also conscious of the potential for publication of new data pertaining to tests included in this review. Whilst not fulfilling the usual Cochrane up-to-date criteria, this review is published because it provides historical context in what is a rapidly-changing field, and because it is unlikely to ever be repeated.