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First trimester ultrasound tests alone or in combination with first trimester serum tests for Down's syndrome screening (Review)

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[Diagnostic Test Accuracy Review]

First trimester ultrasound tests alone or in combination with first trimester serum tests for Down's syndrome screening

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ABSTRACT

Background

Down's syndrome occurs when a person has three, rather than two copies of chromosome 21; or the specific area of chromosome 21 implicated in causing Down's syndrome. It is the commonest congenital cause of mental disability and also leads to numerous metabolic and structural problems. It can be life-threatening, or lead to considerable ill health, although some individuals have only mild problems and can lead relatively normal lives. Having a baby with Down's syndrome is likely to have a significant impact on family life.

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 and false negative screening tests (i.e. invasive diagnostic testing, and the possibility that a miscarried fetus may be chromosomally normal). 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 trimester ultrasound markers alone, and in combination with first trimester serum tests for the detection of Down's syndrome.

Search methods

We carried out extensive literature searches including 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), and The Database of Abstracts of Reviews of Effects (the Cochrane Library 2011, Issue 7). We checked reference lists and published review articles for additional potentially relevant studies.

Selection criteria

Studies evaluating tests of first trimester ultrasound screening, alone or in combination with first trimester serum tests (up to 14 weeks' gestation) for Down's syndrome, 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

We included 126 studies (152 publications) involving 1,604,040 fetuses (including 8454 Down's syndrome cases). Studies were generally good quality, although differential verification was common with invasive testing of only high-risk pregnancies. Sixty test combinations were evaluated formed from combinations of 11 different ultrasound markers (nuchal translucency (NT), nasal bone, ductus venosus Doppler, maxillary bone length, fetal heart rate, aberrant right subclavian artery, frontomaxillary facial angle, presence of mitral gap, tricuspid regurgitation, tricuspid blood flow and iliac angle 90 degrees); 12 serum tests (inhibin A, alpha-fetoprotein (AFP), free beta human chorionic gonadotrophin (ßhCG), total hCG, pregnancy-associated plasma protein A (PAPP-A), unconjugated oestriol (uE3), disintegrin and metalloprotease 12 (ADAM 12), placental growth factor (PlGF), placental growth hormone (PGH), invasive trophoblast antigen (ITA) (synonymous with hyperglycosylated hCG), growth hormone binding protein (GHBP) and placental protein 13 (PP13)); and maternal age. The most frequently evaluated serum markers in combination with ultrasound markers were PAPP-A and free ßhCG.

Comparisons of the 10 most frequently evaluated test strategies showed that a combined NT, PAPP-A, free ßhCG and maternal age test strategy significantly outperformed ultrasound markers alone (with or without maternal age) except nasal bone, detecting about nine out of every 10 Down's syndrome pregnancies at a 5% false positive rate (FPR). In both direct and indirect comparisons, the combined NT, PAPP-A, free ßhCG and maternal age test strategy showed superior diagnostic accuracy to an NT and maternal age test strategy ($P < 0.0001$). Based on the indirect comparison of all available studies for the two tests, the sensitivity (95% confidence interval) estimated at a 5% FPR for the combined NT, PAPP-A, free ßhCG and maternal age test strategy (69 studies; 1,173,853 fetuses including 6010 with Down's syndrome) was 87% (86 to 89) and for the NT and maternal age test strategy (50 studies; 530,874 fetuses including 2701 Down's syndrome pregnancies) was 71% (66 to 75). Combinations of NT with other ultrasound markers, PAPP-A and free ßhCG were evaluated in one or two studies and showed sensitivities of more than 90% and specificities of more than 95%.

High-risk populations (defined before screening was done, mainly due to advanced maternal age of 35 years or more, or previous pregnancies affected with Down's syndrome) showed lower detection rates compared to routine screening populations at a 5% FPR. Women who miscarried in the over 35 group were more likely to have been offered an invasive test to verify a negative screening results, whereas those under 35 were usually not offered invasive testing for a negative screening result. Pregnancy loss in women under 35 therefore leads to under-ascertainment of screening results, potentially missing a proportion of affected pregnancies and affecting test sensitivity. Conversely, for the NT, PAPP-A, free ßhCG and maternal age test strategy, detection rates and false positive rates increased with maternal age in the five studies that provided data separately for the subset of women aged 35 years or more.

Authors' conclusions

Test strategies that combine ultrasound markers with serum markers, especially PAPP-A and free ßhCG, and maternal age were significantly better than those involving only ultrasound markers (with or without maternal age) except nasal bone. They detect about nine out of 10 Down's affected pregnancies for a fixed 5% FPR. Although the absence of nasal bone appeared to have a high diagnostic accuracy, only five out of 10 affected Down's pregnancies were detected at a 1% FPR.

PLAIN LANGUAGE SUMMARY

Screening tests for Down's syndrome in 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 during pregnancy to assist them in making decisions. If a mother is carrying a baby with Down's, 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 Down's child.

The most accurate tests for Down's 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. Both these tests involve inserting needles through the mother's abdomen and are known to increase the risk of miscarriage. Thus the tests are not suitable for offering to 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, they can miss cases of Down's and also give a 'high risk' test results to a number of women whose babies are not affected by Down's. Thus pregnancies identified as 'high risk' using these screening tests require further testing using amniocentesis (from 15 weeks' gestation) or CVS (from 10 + 0 to 13 + 6 weeks' gestation) to confirm a diagnosis of Down's.

What we did

The aim of this review was to find out which of the first trimester ultrasound screening tests, with or without first trimester serum tests done during the first 14 weeks of pregnancy are the most accurate at predicting the risk of a pregnancy being affected by Down's. We looked at 11 different ultrasound markers and 12 different serum markers that can be used alone, in ratios or in combination, taken before 14 weeks' gestation, thus creating 60 screening tests for Down's. We found 126 studies, involving 1,604,040 fetuses (including 8454 fetuses affected by Down's syndrome).

What we found

For the first 14 weeks of pregnancy, the evidence supports the use of first trimester ultrasound tests in combination with two serum (blood) markers - especially pregnancy-associated plasma protein A (PAPP-A) and free beta human chorionic gonadotrophin (ßhCG) - and maternal age, for Down's syndrome screening. In general, these tests are better than ultrasound markers on their own. They detect nine out of 10 pregnancies affected by Down's syndrome. Five per cent of women undertaking the test will have a high risk test result, however the majority of these pregnancies will not be affected by Down's syndrome.

Other important information to consider

The ultrasound tests themselves have no adverse effects for the woman, 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.

Summary of findings 1. Performance of the 10 most evaluated first trimester ultrasound markers alone or in combination with first trimester serum tests

Review question	What is the accuracy of ultrasound based markers alone and in combination with maternal age and/or first trimester serum markers for screening for Down's syndrome?
Population	Pregnant women at less than 14 weeks' gestation confirmed by ultrasound, who had not undergone previous testing for Down's syndrome. Some studies were undertaken in women identified to be at high risk based on maternal age.
Settings	All settings.
Numbers of studies, pregnancies and Down's syndrome cases	126 studies (reported in 152 publications) involving 1,604,040 fetuses of which 8454 were Down's syndrome cases
Index tests	Risk scores computed using maternal age and first trimester ultrasound and serum markers for ultrasound markers - NT, nasal bone, ductus venosus Doppler, maxillary bone length, fetal heart rate, aberrant right subclavian artery, frontomaxillary facial angle, presence of mitral gap, tricuspid regurgitation, tricuspid blood flow and iliac angle 90 degrees - and serum markers - inhibin A, AFP, free ßhCG, total hCG, PAPP-A, uE3, ADAM 12, PlGF, PGH, ITA (h-hCG), GHBP and PP13.
Reference standards	Chromosomal verification (amniocentesis and CVS undertaken during pregnancy, and postnatal karyotyping) and postnatal macroscopic inspection.
Study limitations	116 studies only used selective chromosomal verification during pregnancy, and were at risk of under-ascertainment of Down's syndrome cases due to pregnancy loss between administering the serum test and the reference standard.

Test strategy	**Studies**	**Women (Down's cases)**	**Sensitivity (95% CI)**	**Specificity (95% CI)***	**Consequences in a hypothetical cohort of 10,000 pregnant women assuming Down's syndrome affects approximately one in 800 live-born babies**	
					Missed cases	**False positives**
Nasal bone	11	48,279 (290)	49 (34, 64)	99 (99, 100)	7	100
NT	13	90,978 (593)	70 (61, 78)	95	4	500
NT and maternal age	50	530,874 (2701)	71 (66, 75)	95	4	500
Nasal bone and maternal age	4	25,303 (165)	68 (28, 92)	95	4	500

Ductus and maternal age	5	5331 (165)	68 (49, 83)	95	4	500
NT, nasal bone and maternal age	5	29,699 (221)	78 (55, 91)	95	3	500
NT, free ßhCG and maternal age	5	10,795 (421)	77 (72, 82)	95	3	500
NT, PAPP-A and maternal age	5	9814 (372)	81 (75, 86)	95	3	500
NT, PAPP-A, free ßhCG and maternal age	69	1,173,853 (6010)	87 (86, 89)	95	2	500
NT, PAPP-A, free ßhCG, ADAM 12 and maternal age	4	2571 (256)	82 (75, 87)	95	3	500

*We estimated sensitivity (with a 95% confidence interval) at a 5% false positive rate from the summary ROC curve obtained for each test except nasal bone. For nasal bone, the pooled specificity is reported because the cut-point was absence or presence of nasal bone, and all studies reported false positive rates below 5% so estimation of sensitivity at a fixed 5% FPR was not appropriate.

Summary of findings 2. Performance of other first trimester ultrasound markers alone or in combination with first trimester serum tests

Test strategy	Studies	Women (Down's cases)	Sensitivity* (95% CI)	Specificity* (95% CI)	Threshold
Without maternal age					
Ultrasound markers alone					
Aberrant right subclavian artery	1	425 (51)	8 (2, 19)	99 (98, 100)	Feature
Frontomaxillary facial angle	1	242 (22)	18 (5, 40)	98 (95, 99)	> 95th percentile
Presence of mitral gap	1	217 (20)	20 (6, 44)	87 (81, 91)	Feature
Maxillary bone length	1	927 (88)	24 (15, 34)	95 (93, 96)	5th centile
Tricuspid regurgitation	1	312 (20)	50 (27, 73)	98 (96, 99)	Feature
Iliac angle 90 degrees	1	2032 (52)	60 (45, 73)	98 (97, 98)	Feature

Ductus venosus a-wave reversed	1	378 (72)	68 (56, 79)	70 (64, 75)	Feature
Ductus venosus pulsivity index	1	378 (72)	81 (70, 89)	58 (52, 63)	> 95th percentile
NT and nasal bone	1	486 (38)	89 (75, 97)	93 (91, 95)	Absent nasal bone and NT ≥ 95th centile
Ultrasound and double serum markers					
NT, free ßhCG and PAPP-A	1	6508 (40)	90 (76, 97)	95 (95, 96)	First trimester incidence rate 63.3%
With maternal age					
Ultrasound markers alone					
NT-adjusted risk > 1:300 and abnormal ductus venosus flow and absent nasal bones	1	544 (47)	21 (11, 36)	100 (99, 100)	1:300 risk
NT and ductus	3	23,697 (177)	76 to 93	73 to 99	5% FPR, 1:250 risk, feature
NT and tricuspid blood flow	1	19,736 (122)	85 (78, 91)	97 (97, 98)	1:100 risk
Ultrasound and single serum markers					
NT and inhibin A	2	1150 (97)	61 to 75	95 to 96	5% FPR, 1:250 risk
NT and AFP	1	1110 (85)	61 (50, 72)	95 (94, 96)	5% FPR
NT and total hCG	1	1110 (85)	61 (50, 72)	95 (94, 96)	5% FPR
NT and ITA	1	278 (54)	80 (66, 89)	95 (91, 98)	5% FPR
Ultrasound and double serum markers					
NT, AFP and free ßhCG	2	2766 (90)	66 to 100	93 to 95	5% FPR, 1:250 risk
NT, PAPP-A and inhibin A	2	1150 (97)	80 to 83	95 to 96	5% FPR, 1:250 risk
NT, total hCG and inhibin A	1	1110 (85)	62 (51, 73)	95 (94, 96)	5% FPR

NT, free ßhCG and inhibin A	1	1110 (85)	66 (55, 76)	95 (94, 96)	5% FPR
NT, free ßhCG and ADAM 12	1	351 (31)	68 (49, 83)	95 (92, 97)	5% FPR
NT, PAPP-A and uE3	1	576 (24)	79 (58, 93)	95 (93, 97)	5% FPR
NT, total hCG and PAPP-A	1	1110 (85)	80 (70, 88)	95 (94, 96)	5% FPR
NT, AFP and PAPP-A	1	1110 (85)	80 (70, 88)	95 (94, 96)	5% FPR
NT, PAPP-A and ITA	2	11,053 (77)	83 (73, 90)	95	5% FPR
NT, PAPP-A and ADAM 12	2	1042 (77)	83 (73, 90)	95	5% FPR
Free ßhCG and PAPP-A, if risk between 1:42 and 1:1000 (intermediate risk), NToffered, final composite risk !:250	1	10,189 (44)	89 (75, 96)	94 (94, 95)	1:250 risk
NT, ductus, free ßhCG and PAPP-A	3	30,061 (212)	83 to 96	97 to 99	1:100 risk, 1:250 risk
NT, nasal bone, free ßhCG and PAPP-A	3	41,842 (271)	89 to 94	95 to 98	5% FPR, 1:100 risk, 1:300 risk
NT, PAPP-A, free ßhCG and ductus venosus pulsivity index	1	7,250 (66)	89 (79, 96)	95 (94, 95)	5% FPR
NT, tricuspid blood flow, free ßhCG and PAPP-A	1	19,736 (122)	91 (84, 95)	97 (97, 98)	1:100 risk
NT, fetal heart rate, free ßhCG and PAPP-A	2	76,385 (517)	92 (89, 94)	95	5% FPR
NT, fetal heart rate, nasal bone, free ßhCG and PAPP-A	1	19,736 (122)	95 (90, 98)	96 (95, 96)	1:200 risk
NT, fetal heart rate, tricuspid blood flow, free ßhCG and PAPP-A	1	19,736 (122)	96 (91, 99)	95 (95, 95)	5% FPR
NT, fetal heart rate, ductus, free ßhCG and PAPP-A	1	19,614 (122)	97 (92, 99)	95 (95, 95)	5% FPR
Ultrasound and triple serum markers					
NT, AFP, free ßhCG and PAPP-A	3	6789 (135)	73 to 84	95	5% FPR, 1:250 risk
NT, PAPP-A, free ßhCG and PP13	1	998 (151)	77 (69, 83)	95 (93, 96)	5% FPR
NT, PAPP-A, free ßhCG and total hCG	1	998 (151)	77 (69, 83)	95 (93, 96)	5% FPR

NT, total hCG, inhibin A and PAPP-A	1	1110 (85)	81 (71, 89)	95 (94, 96)	5% FPR
NT, free ßhCG, inhibin A and PAPP-A	1	1110 (85)	84 (74, 91)	95 (94, 96)	5% FPR
NT, PAPP-A, free ßhCG and PGH	1	335 (74)	86 (77, 93)	95 (92, 97)	5% FPR
NT, PAPP-A, free ßhCG and PlGF	2	1443 (221)	88 (70, 95)	95	5% FPR
NT, PAPP-A, free ßhCG and GHBP	1	335 (74)	91 (81, 96)	95 (92, 97)	5% FPR
Ultrasound and quadruple serum markers					
NT, PAPP-A, free ßhCG, ADAM 12 and PlGF	1	998 (151)	79 (72, 86)	95 (93, 96)	5% FPR
Ultrasound and quintuple serum markers					
NT, PAPP-A, free ßhCG, ADAM 12, total hCG and PlGF	1	998 (151)	79 (72, 86)	95 (93, 96)	5% FPR
NT, total hCG, inhibin A, PAPP-A, AFP and uE3	1	1110 (85)	84 (74, 91)	95 (94, 96)	5% FPR
NT, free ßhCG, inhibin A, PAPP-A, AFP and uE3	1	1110 (85)	86 (77, 92)	95 (94, 96)	5% FPR
Ultrasound and sextuple serum markers					
NT, PAPP-A, free ßhCG, ADAM 12, total hCG, PlGF and PP13	1	998 (151)	80 (73, 86)	95 (93, 96)	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 meta-analysis was not performed because there were only two or three studies and no common threshold.

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 1987). 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 or translocation. 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 1984). 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 subunit of hCG 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).

Two other serum markers, produced by the placenta, have been linked with Down's syndrome, namely pregnancy-associated plasma protein A or PAPP-A, and Inhibin A. 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 and hence reliability of this marker, and the effect this will have on individual risk.

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 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 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.

Index test(s)

This review examined ultrasound and serum screening tests used in the first trimester of pregnancy (up to 14 weeks' gestation). The tests included the following individual ultrasound markers: nuchal translucency (NT), nasal bone, ductus venosus Doppler, maxillary bone length, fetal heart rate, aberrant right subclavian artery, frontomaxillary facial angle, presence of mitral gap, tricuspid regurgitation, tricuspid blood flow and iliac angle 90 degrees; and the following individual serum markers: inhibin A, AFP, free ßhCG, total hCG, pregnancy-associated plasma protein A (PAPP-A), uE3, a disintegrin and metalloprotease 12 (ADAM 12), placental growth factor (PlGF), placental growth hormone (PGH) invasive trophoblast antigen (ITA) (synonymous with hyperglycosylated hCG), growth hormone binding protein (GHBP) and placental protein 13 (PP13).

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.

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. These tests are considered to be reference tests rather than index or screening tests. 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. 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. Amniocentesis and CVS are both methods of obtaining fetal chromosome 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). A more recent systematic review suggests that the procedure-related risk of pregnancy loss is lower than this (Akolekar 2015).

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 for this review, and were not included in the search strategy, which preceded their widespread introduction. A systematic review conducted by another group is currently in preparation, examining this newer screening technology (Badeau 2015).

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 tests that combine markers from the first trimester with markers from the second trimester (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).

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 provide 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.

A systematic review of second trimester ultrasound markers in the detection of Down’s syndrome fetuses was published in 2001 which concluded that nuchal fold thickening may be useful in detecting Down’s syndrome, but that it was not sensitive enough to use as a screening test. The review concluded that the other second trimester ultrasound markers did not usefully distinguish between Down’s syndrome and pregnancies without Down’s syndrome (Smith-Bindman 2001). There has yet to be a systematic review and meta-analysis of the observed data on serum, urine and first trimester ultrasound markers, in order to draw rigorous and robust conclusions about the diagnostic accuracy of available Down’s syndrome screening tests.

OBJECTIVES

The aim of this review was to estimate and compare the accuracy of first trimester ultrasound with and without serum markers for the detection of Down’s syndrome in the antenatal period, both

as individual markers and as combinations of markers. Accuracy is described by the proportion of fetuses with Down's syndrome detected by screening before birth (sensitivity or detection rate) and the proportion with a low-risk screening test result (negative) from amongst babies born without Down's syndrome. We grouped our analyses to focus on investigating the value of adding increasing numbers of markers (comparing single, dual, triple, quadruple, quintuple and sextuple tests).

Investigation of sources of heterogeneity

We had 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 (multifetal) pregnancy, diabetes and family history of Down's syndrome. We also planned to examine whether there was 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 at less than 14 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, multifetal pregnancy, diabetes mellitus and a family history of Down's syndrome.

Index tests

Improved diagnostic performance can be obtained by using several tests in combination, such as maternal age and serum marker combinations, or combinations of maternal age, serum markers and sonographic measurements. We examined individual first trimester ultrasound markers or combinations of these markers with one or more first trimester serum tests, with and without adjustment for maternal age.

The following ultrasound markers were examined: NT, nasal bone, ductus venosus Doppler, maxillary bone length, fetal heart rate, aberrant right subclavian artery, frontomaxillary facial angle, presence of mitral gap, tricuspid regurgitation, tricuspid blood flow and iliac angle 90 degrees.

The serum markers examined in different combinations with ultrasound markers were inhibin A, AFP, free ßhCG, total hCG, PAPP-A, uE3, ADAM 12, PlGF, PGH, ITA (h-hCG), GHBP and PP13.

We examined comparisons of ultrasound markers in isolation and in various combinations with or without serum markers. The combinations included one or two ultrasound markers with single (one marker), double (two markers), triple (three markers), quadruple (four markers), quintuple and sextuple (six markers) serum markers, with or without adjustment for maternal age.

Where tests were used in combinations, we examined the performance of test combinations according to predicted probabilities computed using risk equations and dichotomised into high risk and low risk at some standard high-risk value. Risk equations are often coded into software to produce 'risk score' computations, which provide an individual's predicted probability of Down's syndrome.

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 syndrome, or on 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 urine 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

Electronic searches

We applied a sensitive search strategy to search the following databases using the search strategies listed in Appendix 1. We used one generic search to identify studies for all reviews in this series.

We searched the following databases

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 Effects (the Cochrane Library 2011, Issue 7)
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 did not apply a diagnostic test filter, and we did not apply language restrictions to the search.

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

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, multifetal 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 review 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, multifetal 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 restricting to studies which 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 (historically reported in literature based on age-related risk) 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. When there was an insufficient number of studies to reliably estimate all the parameters in the HSROC model, univariate random-effects logistic regression models were used to obtain pooled estimates of sensitivity and specificity. 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 model since all specificities are the same value. Thus, at a fixed specificity value, the summary estimate of sensitivity was obtained using a univariate random-effects logistic regression 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 for each test from each study to estimate a SROC curve without restricting to a common threshold. The threshold for each test was chosen from 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 most evaluated or best performing 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 very few studies for each test, a symmetric summary ROC curve was assumed. In addition, because the analysis failed to converge, we assumed fixed-effect for the threshold and accuracy parameters. An estimate of the sensitivity of each test for a 5% FPR was derived from the SROC 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 simplified HSROC model with fixed-effect and symmetrical underlying SROC curves because the number of studies was insufficient to estimate between study heterogeneity in accuracy and threshold or asymmetry in the shape of the SROC 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 ratios of 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 had planned to investigate heterogeneity by adding covariate terms to the HSROC model (meta-regression) to assess the effect of each factor stated in the Investigation of sources of heterogeneity section on accuracy and threshold.

Sensitivity analyses

Mothers with pregnancies identified as high risk for Down's syndrome by ultrasound and serum testing were often offered immediate definitive testing by amniocentesis, whereas those considered low risk were 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 potential bias introduced by such a mechanism, where possible, we performed sensitivity analyses by increasing the number of false negatives in studies where delayed verification in test negatives occurred (Mol 1999). We increased the number of false negatives in such studies by a multiplicative factor that we applied incrementally from 10% to 50%. The final value of 50% assumes the true number of false negatives is 1.5 times the

observed number of false negatives, implying the observed number of false negatives.is 67% (i.e. 1/1.5) of the true number and the fetal loss rate is 33%. Since no increments were added to the number of true negatives, this represents a scenario where a third more pregnancies affected by Down's syndrome is likely to miscarry compared to those unaffected by Down's syndrome. This is thought to be higher than the likely value.

We intended to conduct these sensitivity analyses on analyses investigating the effect of maternal age on test sensitivity. However, due to limited data, we performed the sensitivity analyses when comparing high-risk populations with routine screening populations. This comparison was considered a proxy for the effect of maternal age because the main indication for referral for invasive testing was often increased risk due to advanced maternal age.

RESULTS

Results of the search

After the results from each bibliographic database were combined and duplicates were removed, the search for the whole suite of reviews identified a total of 15,394 papers. After screening out obviously inappropriate 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 126 studies (reported in 152 publications) were included in this review of first trimester ultrasound alone or in combination with first trimester serum screening. Since women with multifetal pregnancies were included in six of the 126 studies, where a study included multifetal pregnancies, we report fetuses rather than women or pregnancies. The review involved 1,604,040 fetuses including 8454 Down's syndrome cases.

A total of 60 different test strategies were evaluated in the 126 studies. These tests were formed from combinations of different ultrasound markers, serum tests and maternal age. The 11 individual ultrasound markers were nuchal translucency (NT), nasal bone, ductus venosus Doppler (ductus venosus a-wave reversed, ductus venosus pulsivity index), maxillary bone length, fetal heart rate, aberrant right subclavian artery, frontomaxillary facial angle, presence of mitral gap, tricuspid regurgitation, tricuspid blood flow and iliac angle 90 degrees. The 12 individual serum markers were inhibin A, alpha-fetoprotein (AFP), free beta human chorionic gonadotrophin (ßhCG), total hCG, pregnancy-associated plasma protein A (PAPP-A), unconjugated oestriol (uE3), disintegrin and metalloprotease 12 (ADAM 12), placental growth factor (PlGF), placental growth hormone (PGH), invasive trophoblast antigen (ITA) (h-hCG), growth hormone binding protein (GHBP), and placental protein 13 (PP13). The strategies evaluated, with or without maternal age, included 13 single ultrasound markers; five combinations of two or more ultrasound markers; six ultrasound and single serum marker combinations; 22 ultrasound and double serum marker combinations; nine ultrasound and triple serum marker combinations; one ultrasound and quadruple serum marker combination; three ultrasound and quintuple serum marker combinations; and one ultrasound and sextuple serum marker combination. Seventy-eight of the 126 studies only evaluated the performance of a single first trimester ultrasound or ultrasound and serum test or test strategy; 27 studies evaluated two tests, 10 evaluated three tests, four evaluated four tests, four evaluated five tests, one evaluated eight tests (Koster 2011), one evaluated 11 tests (Kagan 2010), and one evaluated 19 tests (Wald 2003).

The following test combinations were evaluated by four or more studies.

Ultrasound and triple serum markers

- NT, PAPP-A, free ßhCG, ADAM 12 and maternal age (four studies; 2571 women, including 256 Down's syndrome pregnancies)

Ultrasound and double serum markers

- NT, PAPP-A, free ßhCG and maternal age (69 studies; 1,173,853 fetuses, including 6010 Down's syndrome cases)

Ultrasound and single serum markers

- NT, free ßhCG and maternal age (five studies; 10,795 women, including 421 Down's syndrome pregnancies)
- NT, PAPP-A and maternal age (five studies; 9,814 women including 372 Down's syndrome pregnancies)

Ultrasound markers alone

- NT, nasal bone and maternal age (five studies, 29,699 women, including 221 Down's syndrome pregnancies)
- NT and maternal age (50 studies; 530,874 fetuses including 2701 Down's syndrome cases)
- Nasal bone and maternal age (four studies; 25,303 women, including 165 Down's syndrome pregnancies)
- Ductus and maternal age (five studies; 5,331 women including 165 Down's syndrome pregnancies)
- Nasal bone (11 studies; 48,279 fetuses including 290 Down's syndrome cases)
- NT (13 studies; 90,978 fetuses, including 593 Down's syndrome cases)

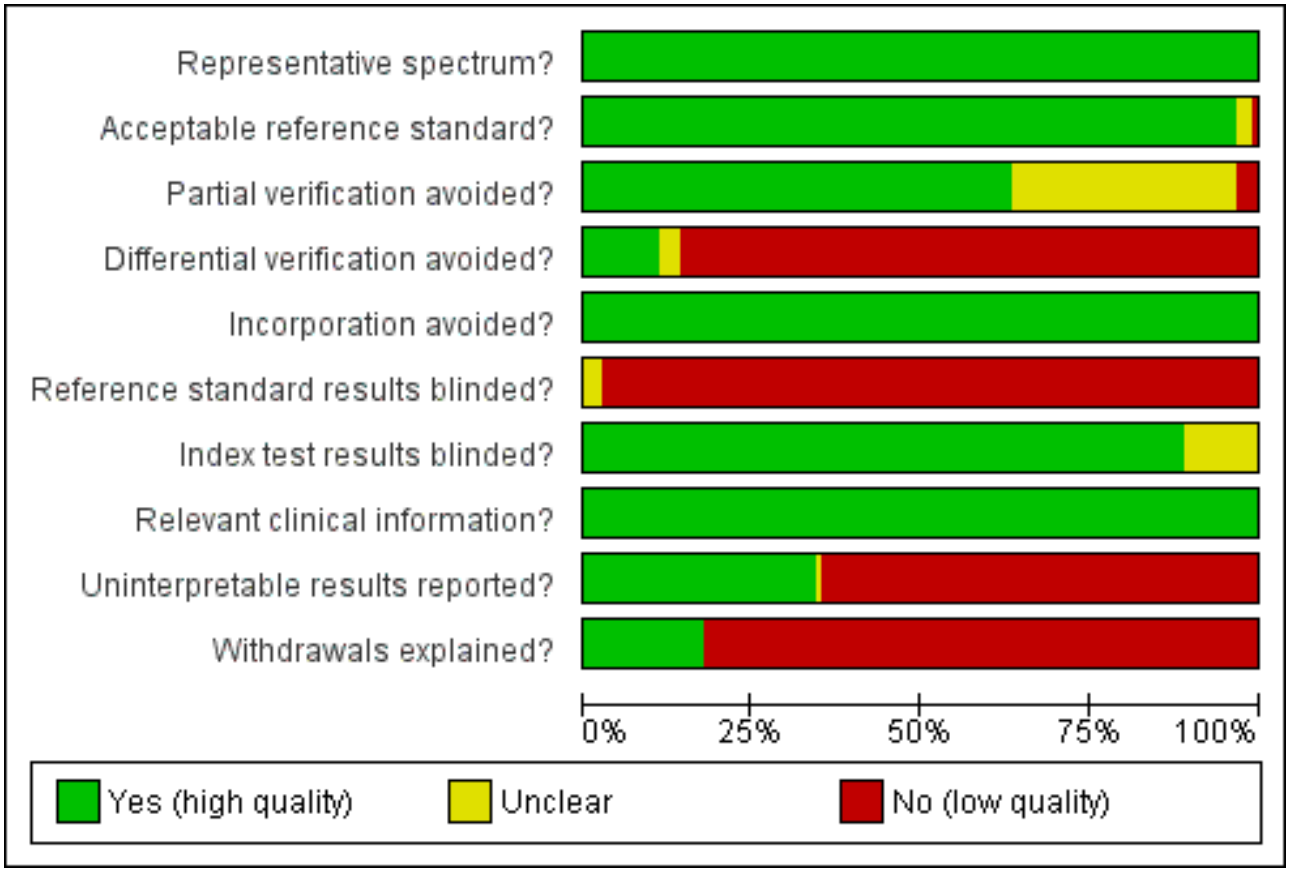
Of the remaining test combinations, four were evaluated in three studies, six were evaluated in two studies and the remaining 40 in single studies only.

Methodological quality of included studies

The studies were judged to be of high methodological quality in most categories (Figure 1) and details are provided in the Characteristics of included studies. The spectrum of participants was judged to be representative in all study cohorts. The reference standard used was judged unclear in three studies (Hafner 1998; Krantz 2000; Orlandi 1997) and unacceptable in one study (Noble 1995). 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 (negative) will have their screening test verified at birth, rather than by invasive diagnosis in the antenatal period. Partial verification was avoided in 81 study cohorts (64%) and differential verification was avoided in 15 study cohorts (12%). Both differential and partial verification was avoided in 14 study cohorts (Biagiotti 1998; Borenstein 2008; Christiansen 2005; Cicero 2004a; De Graaf 1999; Hewitt 1996; Maiz 2007; Matias 1998; Matias 2001; Mavrides 2002; Molina 2010 high risk; Otaño 2002; Pajkrt 1998a; Prefumo 2005). Of the 14 study cohorts, the populations in 13 were high-risk referral for invasive testing (prior to screening being undertaken),

while one (Christiansen 2005) obtained maternal serum samples through screening programmes for syphilis and Down's syndrome. Reference standard results were unblinded in 124 study cohorts and unclear in three study cohorts. In contrast, index test results were blinded in 113 study cohorts and unclear in 14. 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.



Most studies seemed to indicate 100% follow-up, however there will inevitably be losses to follow-up due to women moving out of area, for example. Studies sometimes accounted for these and it is unlikely that there were enough losses to follow-up to have introduced significant bias. There was likely 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. There is a higher natural miscarriage rate in the first trimester, however this will be uniform across studies and therefore unlikely to introduce significant bias.

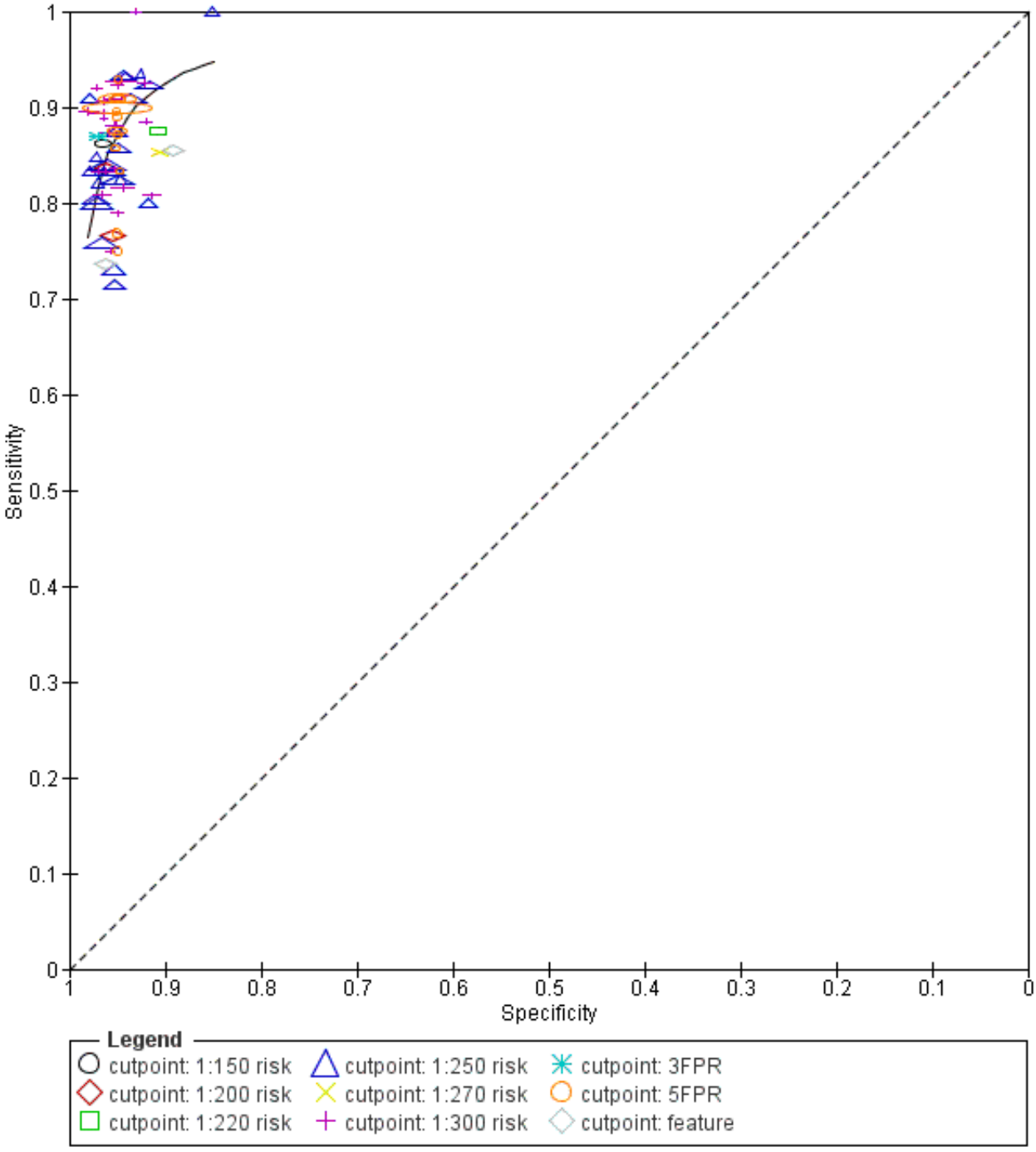
Some studies which provided estimates of risk using multivariable equations used the same data set to evaluate performance of the risk equation as was used to derive the equation. This is often thought to lead to over-estimation of test performance.

Findings

The results for the 10 most evaluated test strategies are presented in Summary of findings 1. Additional information and results at specific thresholds are provided below.

1) NT, PAPP-A, free ßhCG and maternal age (Figure 2 **)**

Figure 2. Study estimates of sensitivity and specificity with a summary ROC curve for the NT, PAPP-A, free ßhCG and maternal age test combination at different cut-points. Each symbol represents a pair of sensitivity and specificity at one cut-point from each study.



This was the most evaluated test strategy and accounted for most (73%) of the fetuses in this systematic review. The test was evaluated by 69 studies and involved 1,173,853 fetuses (including 6010 Down's syndrome cases). Six studies (Cowans 2009; Ekelund 2008; Kagan 2010; Merz 2011; Nicolaides 2005; Wright 2010) contributed more than half the total number of fetuses affected by Down's syndrome (3057); the largest study (Wright 2010) included 223,361 women in whom 886 pregnancies were affected by Down's syndrome. Across the 69 studies, data were presented at 10 cut-points (1% false positive rate (FPR), 3% FPR, 4.5% FPR, 5% FPR, 1:150 risk, 1:200 risk, 1:220 risk, 1:250 risk, 1:270 risk and 1:300 risk). At a cut-point of 5% FPR (24 studies, 391,874 fetuses including 2521

fetuses affected by Down's syndrome), the estimated sensitivity was 87% (95% CI 84 to 89); at a cut-point of 1:250 risk (25 studies; 174,712 fetuses including 1032 fetuses affected by Down's syndrome), the estimated sensitivity was 85% (95% CI 81 to 87) and the specificity was 95% (95% CI 95 to 96).

2) NT, PAPP-A, free ßhCG, ADAM 12 and maternal age

This combination of NT, triple serum markers and maternal age was evaluated by four studies (Christiansen 2010; Koster 2011; Spencer 2008; Torring 2010) and included 2571 women (256 pregnancies were affected by Down's syndrome). Studies presented data for cut-points of 5% FPR (Christiansen 2010; Koster 2011; Spencer 2008; Torring 2010) and 1;250 risk (Christiansen 2010; Torring 2010). At a cut-point of 5% FPR (four studies, 2571 women), the estimated sensitivity was 85% (95% confidence interval (CI) 75 to 91); at a cut-point of 1:250 risk (two studies; 1222 women in whom 74 pregnancies were affected by Down's syndrome), the estimated sensitivity was 86% (95% CI 77 to 93) and the specificity was 97% (95% CI 96 to 98).

3) NT, PAPP-A and maternal age

This test strategy was evaluated by five studies (Biagiotti 1998; Habayeb 2010; Krantz 2000; Spencer 1999; Wald 2003) and involved 9814 women (including 372 Down's syndrome pregnancies). Data were presented at cut-points of 5% FPR (Biagiotti 1998; Spencer 1999; Wald 2003), 1:100 risk (Habayeb 2010) and 1:185 risk (Krantz 2000). Habayeb 2010 estimated a sensitivity of 67% (95% CI 35 to 90) and specificity of 98% (95% CI 97 to 98) at a cut-point of 1:100 risk based on 1507 women in whom 12 pregnancies were affected by Down's syndrome. At a cut-point of 1:185 risk, Krantz 2000 estimated a sensitivity of 82% (95% CI 65 to 93) and specificity of 95% (95% CI 94 to 96) based on 5809 women in whom 33 pregnancies were affected by Down's syndrome. For the three studies (2498 women in whom 327 pregnancies were affected by Down's syndrome) that reported a 5% FPR, the estimated sensitivity was 80% (95% CI 75 to 84).

4) NT, nasal bone and maternal age

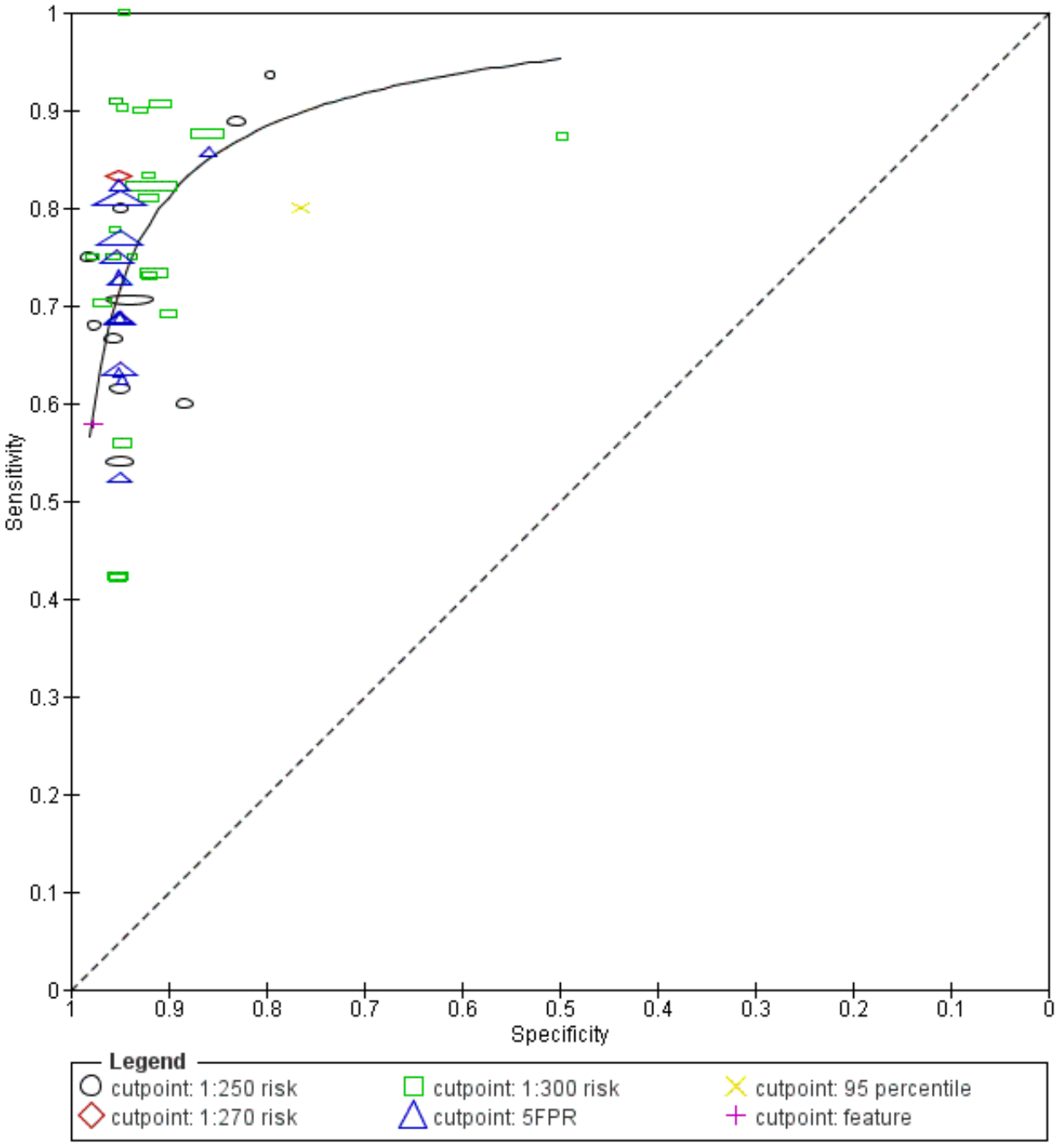
This combination of two ultrasound markers and maternal age was evaluated by five studies (Has 2008; Kagan 2010; Prefumo 2005; Prefumo 2006; Sepulveda 2007) and involved 29,699 women (including 221 Down's syndrome pregnancies). Data were presented at cut-points of 1:100 risk (Kagan 2010) and 1:300 risk (Has 2008; Prefumo 2005; Prefumo 2006; Sepulveda 2007). Kagan 2010 estimated a sensitivity of 83% (95% CI 75 to 89) and specificity of 97% (95% CI 97 to 97) based on 19,736 women in whom 122 pregnancies were affected by Down's syndrome. At a cut-point of 1:300 risk (four studies; 9963 women in whom 99 pregnancies were affected by Down's syndrome), the estimated sensitivity was 61% (95% CI 22 to 89) and the specificity was 97% (95% CI 90 to 99).

5) NT, free ßhCG and maternal age

Results for this combination of NT, a single serum marker and maternal age were obtained from five studies (Biagiotti 1998; Krantz 2000; Noble 1995; Spencer 1999; Wald 2003) involving 10,975 women in whom 421 were affected by Down's syndrome pregnancies. Data were presented at cut-points of 5% FPR (Biagiotti 1998; Noble 1995; Spencer 1999; Wald 2003) and 1:240 risk (Krantz 2000). At a cut-point of 5% FPR (four studies; 4986 women in whom 388 pregnancies were affected by Down's syndrome), the estimated sensitivity was 77% (95% CI 68 to 84). At a cut-point of 1:240 risk, Krantz 2000 estimated a sensitivity of 79% (95% CI 61 to 91) and specificity of 95% (95% CI 94 to 96) based on 5799 women in whom 33 pregnancies were affected by Down's syndrome.

6) NT and maternal age (Figure 3 **)**

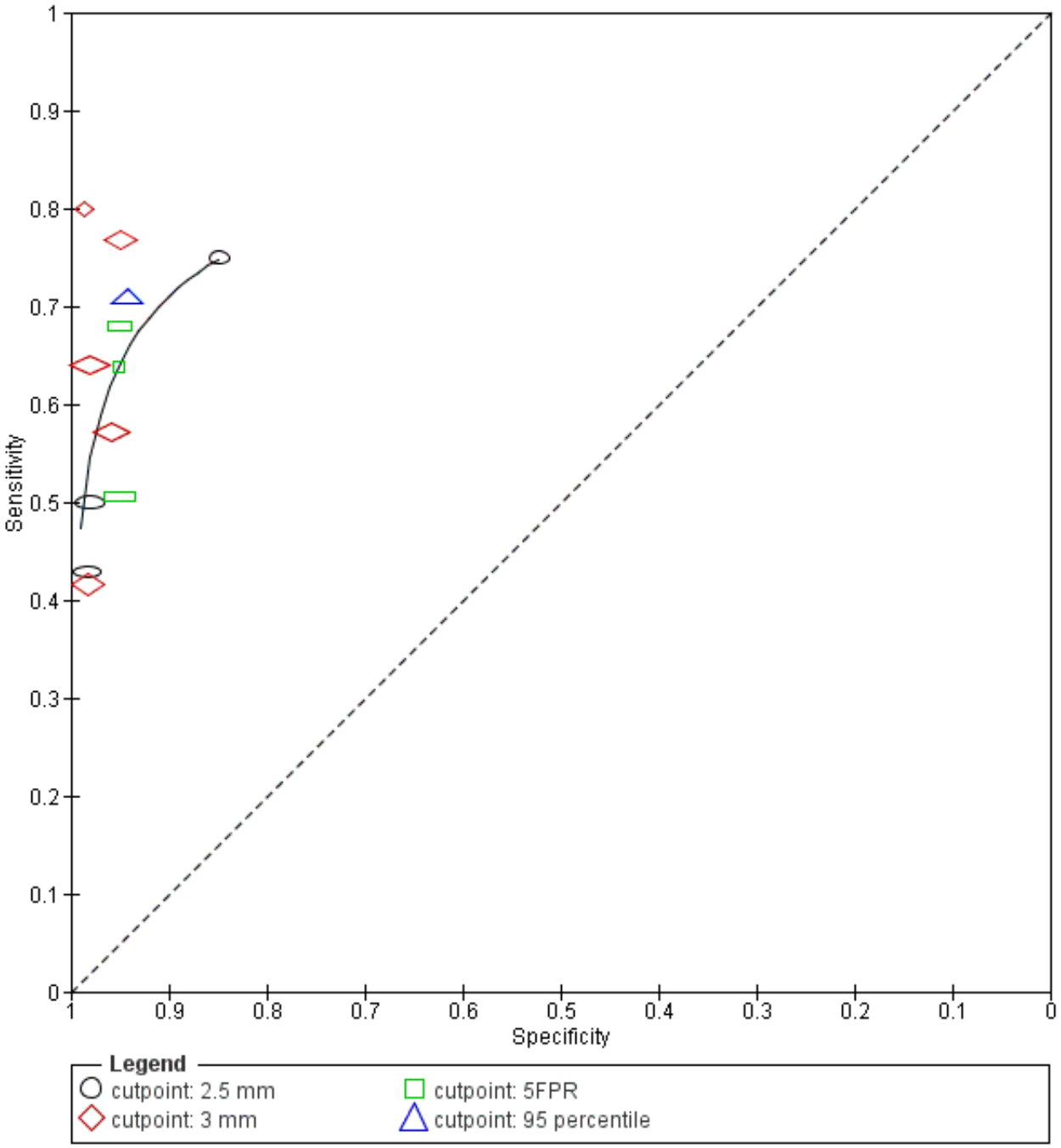
Figure 3. Study estimates of sensitivity and specificity with a summary ROC curve for NT and maternal age across different cut-points. Each symbol represents a pair of sensitivity and specificity at one cut-point from each study.



This ultrasound marker was evaluated in 50 studies that included 530,874 fetuses including 2701 fetuses affected by Down's syndrome. Seven studies (Bestwick 2010; Gasiorek-Wiens 2001; Kagan 2010; O'Leary 2006; Snijders 1998; Wald 2003; Wright 2008) each included over 20,000 fetuses and contributed over half the data (296,481 fetuses including 1444 Down's syndrome cases); Snijders 1998 was the largest study (95,802 fetuses). The 50 studies reported diagnostic accuracy at five different cut-points (1% FPR, 3% FPR, 5% FPR, 1:250 risk and 1:300 risk). At a cut-point of 5% FPR (22 studies; 288,853 fetuses including 1784 Down's syndrome cases), the estimated sensitivity was 71% (95% CI 67 to 75); at a cut-point of 1:250 risk, the estimated sensitivity was 72% (95% CI 62 to 80) and specificity was 94% (95% CI 90 to 96) based on 10 studies of 79,412 fetuses including 247 affected by Down's syndrome.

7) NT (Figure 4 **)**

Figure 4. Study estimates of sensitivity and specificity with a summary ROC curve for NT. Each symbol represents a pair of sensitivity and specificity at one cut-point from each study.



Thirteen studies (Acacio 2001; Babbur 2005; Bestwick 2010; Hafner 1998; Hewitt 1996; Kim 2006; Marsis 2004; Michailidis 2001; Nicolaides 1992; Pajkrt 1998a; Schuchter 2002; Spencer 1999; Wald 2003) evaluated NT in 90,978 fetuses including 593 affected by Down's syndrome. Of the 13 studies, two studies (Bestwick 2010; Wald 2003) had a sample size of more than 20,000 and contributed 69% (62,729 fetuses) of the data. Data were presented at cut-points of 2.5 mm (Acacio 2001; Hafner 1998; Kim 2006; Schuchter 2002), 3 mm (Babbur 2005; Hewitt 1996; Kim 2006; Marsis 2004; Nicolaides 1992; Pajkrt 1998a), 5% FPR (Bestwick 2010; Spencer 1999; Wald 2003) and 99th centile (Michailidis 2001). At a 5% FPR, the estimated sensitivity from the three studies was 62% (95% CI 54 to 69), based on 63,885 fetuses including 401 affected by Down's syndrome. At the 2.5 mm cut-point, the estimated sensitivity from the four

studies was 61% (95% CI 42 to 77) and the specificity was 96% (95% CI 90 to 98) based on 64 affected cases and a total of 11,835 fetuses. For the 3 mm cut-point, the estimated sensitivity from the six studies was 58% (95% CI 48 to 68) and the specificity was 97% (95% CI 96 to 98) based on 136 cases and a total of 10,381 fetuses.

8) Nasal bone and maternal age

Nasal bone adjusted for maternal age was evaluated in four studies (Monni 2005; Prefumo 2005; Prefumo 2006; Viora 2003) involving 25,303 women and included 165 Down's syndrome pregnancies**.** Monni 2005 accounted for 66% (16,641 women) of the data. The estimated summary sensitivity was 49% (95% CI 37 to 60) and the summary specificity was 98% (95% CI 95 to 99).

9) Ductus and maternal age

Five studies (Borrell 2005; Matias 2001; Mavrides 2002; Molina 2010 high risk; Prefumo 2005) evaluated this single ultrasound marker in 5,331 women including 165 Down's syndrome pregnancies. Borrell 2005 contributed 70% (3731 women) of the data. Data were presented at 5% FPR (Borrell 2005; Mavrides 2002), 1:250 risk (Borrell 2005), or fetuses were categorised as negative or positive for Down's syndrome based on normal or abnormal ductus venous flow (Matias 2001; Mavrides 2002; Prefumo 2005). At a 5% FPR, the estimated sensitivity from the two studies was 67% (95% CI 54 to 78) based on 3965 women in whom 55 were affected by Down's syndrome pregnancies.

10) Nasal bone

Results for this single marker were obtained from 11 studies (Cicero 2006; Has 2008; Leung 2009; Malone 2004; Molina 2010 high risk; Moon 2007; Orlandi 2003; Orlandi 2005; Otaño 2002; Ramos-Corpas 2006; Sepulveda 2007) involving 48,279 fetuses including 290 affected by Down's syndrome. Cicero 2006 was the largest study (20,418 women including 140 affected cases), accounting for 42% of the data. The estimated summary sensitivity was 49% (95% CI 34 to 64) and the summary specificity was 99% (95% CI 99 to 100).

11) Other test strategies

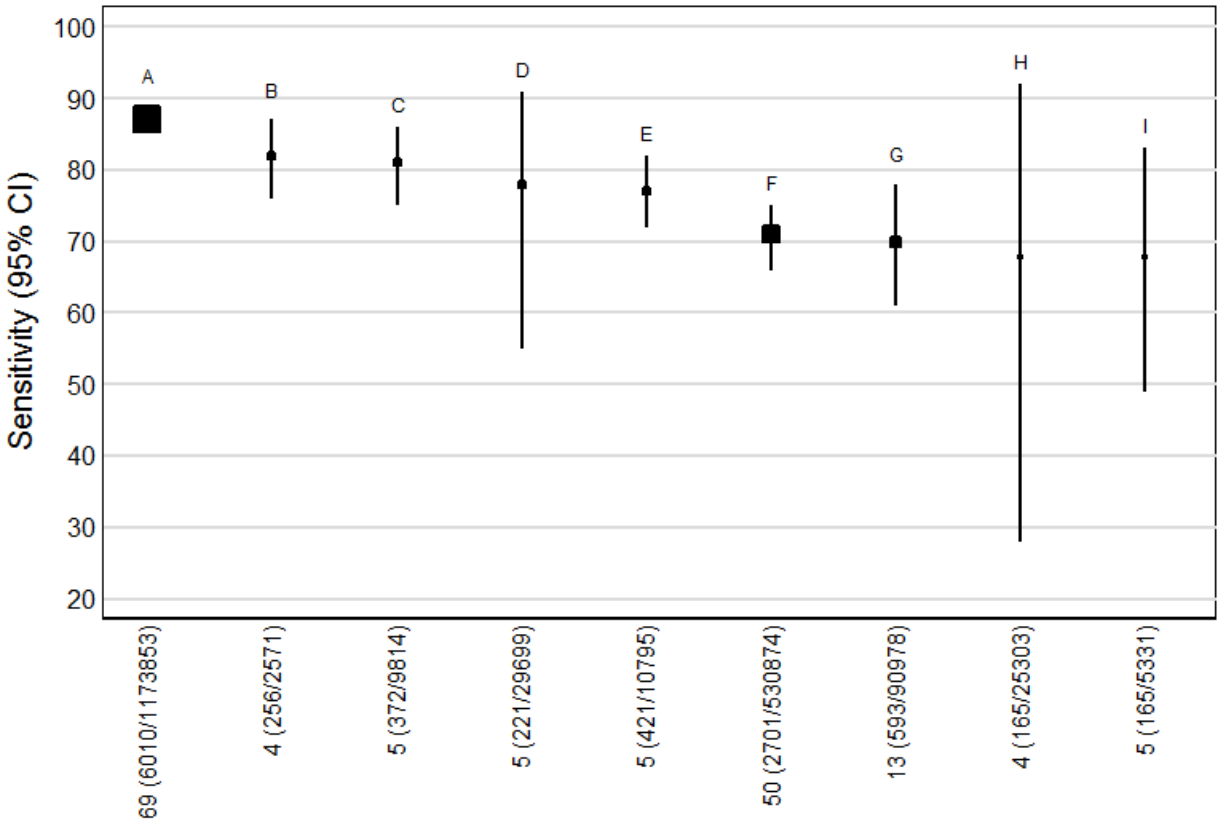
The results for the remaining test strategies are presented in Summary of findings 2. Of the 50 test strategies evaluated in fewer than four studies, 33 test strategies showed estimated sensitivities of at least 70% and estimated specificities of 90%; none of the eight single tests without maternal age achieved this level of test performance. The following seven test strategies evaluated in one or two studies showed sensitivities of more than 90% and specificities of more than 95%.

- **NT, free ßhCG and PAPP-A** evaluated in a single study (Hormansdorfer 2011) estimated a sensitivity of 90% (95% CI 76 to 97%) and specificity of 95% (95% CI 95 to 96) at a first trimester incidence rate of 63.3%.
- **NT, PAPP-A, free ßhCG, GHBP and maternal age** evaluated in a single study (Christiansen 2009) estimated a sensitivity of 91% (95% CI 81 to 96) at a cut-point of 5% FPR.
- **NT, tricuspid blood flow, free ßhCG, PAPP-A and maternal age** evaluated in a single study (Kagan 2010) estimated a sensitivity of 91% (95% CI 84 to 95) and specificity of 97% (95% CI 97 to 98) at a cut-point of 1:100 risk.
- **NT, fetal heart rate, free ßhCG, PAPP-A and maternal age** evaluated in two studies (Kagan 2010; Maiz 2009) estimated a sensitivity of 92% (95% CI 89 to 94) at a cut-point of 5% FPR.
- **NT, fetal heart rate, nasal bone, free ßhCG, PAPP-A and maternal age** evaluated in a single study (Kagan 2010) estimated a sensitivity of 95% (95% CI 90 to 98) and specificity of 96% (95% CI 95 to 96) at a cut-point of 1:200 risk.
- **NT, fetal heart rate, tricuspid blood flow, free ßhCG, PAPP-A and maternal age** evaluated in a single study (Kagan 2010) estimated a sensitivity of 96% (95% CI 91 to 99) at a cut-point of 5% FPR.
- **NT, fetal heart rate, ductus, free ßhCG, PAPP-A and maternal age** evaluated in a single study (Maiz 2009) estimated a sensitivity of 97% (95% CI 92 to 99) at a cut-point of 5% FPR.

Comparative analysis of the 10 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. However, because the 5% FPR was not within the range of the data for the nasal bone marker (the specificities were between 97% and 100%), we did not compute the detection rate at a 5% FPR for this test; the summary sensitivity was 49% (95% CI 34 to 64) and the summary specificity was 99% (95% CI 99 to 100). Figure 5 shows point estimates of the detection rate (and their 95% CIs) at a 5% FPR based on all available data for the remaining nine test strategies; the test strategies are ordered according to decreasing detection rates. The plot shows that for the combined NT, PAPP-A, free ßhCG and maternal age test strategy, the estimated detection rate was 87% (95% CI 86 to 89) based on data from 69 studies with 6010 affected cases out of a total of 1,173,853 participants. The four single ultrasound markers (NT and maternal age; NT; nasal bone and maternal age; and ductus and maternal age) showed the worst performance, whereas, the three test strategies containing PAPP-A showed the highest performance with detection rates above 80%. However, it should be noted that the confidence intervals around the estimates generally overlap though the confidence interval for the combined NT, PAPP-A, free ßhCG and maternal age test strategy is very narrow and not overlapped by five of the other test strategies.

Figure 5. Detection rates (% sensitivity) at a 5% false positive rate for nine of the most evaluated first trimester ultrasound markers alone or in combination with first trimester serum tests. A = NT, PAPP-A, free ßhCG and maternal age; B = NT, PAPP-A, free ßhCG, ADAM 12 and maternal age; C = NT, PAPP-A and maternal age; D = NT, nasal bone and maternal age; E= NT, free ßhCG and maternal age; F= NT and maternal age; G = NT; H = Nasal bone and maternal age; and I = Ductus and maternal age. Each square represents the summary sensitivity for a test strategy at a 5% false positive rate. The size of each square 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. For each test strategy, the number of included studies, Down's syndrome cases and pregnancies are shown on the horizontal axis.



The strength of evidence for differences in the diagnostic performance of the 10 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% CIs and P values for each test comparison. The diagnostic accuracy of NT (with or without maternal age) alone tended to be inferior unlike when combined with serum tests (PAPP-A and free ßhCG). However, all comparisons in this table, except for the combined NT, PAPP-A, free ßhCG and maternal age versus NT and maternal age test comparison (25 studies), were based on five or fewer 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 nasal bone outperformed other ultrasound markers and had similar accuracy with strategies comprising NT and serum markers. Nasal bone was the best performing ultrasound marker (DOR (95% CI): 132 (71 to 245)), and the combined NT, PAPP-A, free ßhCG and maternal age test strategy was the best performing ultrasound and serum test combination (DOR (95% CI): 133 (114 to 155)). Both tests had a much higher diagnostic accuracy than the other tests, and the difference in accuracy was statistically significant in several comparisons especially when compared with single ultrasound markers with or without maternal age. The difference in accuracy between the nasal bone marker and test strategies that included at least one serum test was statistically significant ($P = 0.04$) for only the comparison with the combined NT, free ßhCG and maternal age test strategy. There were no statistically significant differences in accuracy between combinations that included nasal bone and NT

with or without maternal age, and test strategies that included both NT and one or more serum markers. However, these comparisons are potentially confounded by differences between the studies.

Investigation of heterogeneity and sensitivity analyses

We explored the effect of advanced maternal age (< 35 years versus ≥ 35 years) on test performance. However, we were unable to use meta-regression to formally investigate the effect of advanced maternal age due to limited data. Of the 126 included studies, 13 did not report maternal age. The available data for all studies are summarised in Table 3 which also shows the four test combinations (NT, PAPP-A, free ßhCG and maternal age; NT and maternal age; nasal bone alone; and NT alone) that included 10 or more studies. Two studies included only pregnant women with maternal age of 35 years or more; one study (Centini 2005) evaluated the NT, PAPP-A, free ßhCG and maternal age test combination and the other study (Marsis 2004) evaluated NT. Across the four tests there were 12 studies of women considered high-risk referrals; one of the studies (Centini 2005), included only pregnant women ≥ 35 years old. The main indication for referral for invasive testing was often increased risk due to advanced maternal age and so we compared high-risk populations with routine screening populations. The analysis was not performed for nasal bone because only two of the 11 studies were conducted in high-risk populations. The results of the investigation for the remaining three tests together with the sensitivity analyses inflating the false negatives from 10% to 50% in studies where delayed verification in test negatives occurred are shown in Table 4.

Delayed verification was not common in high-risk referral studies as women tended to be offered invasive testing on the basis of the increased risk, and the corrections to the false negatives made very little or no difference to the estimates of sensitivity. However, in screening populations the correction reduced sensitivity, and consequently reduced the apparent relationship between type of population and test performance, observed through the ratio of DORs approaching one. Up to an increase of 40% in the false negatives, the difference in sensitivity between high risk and screening populations for the NT and maternal age test strategy remained statistically significant; the magnitude of the difference dropping from 25% to 17%. However, it should be noted that there were few high-risk referral studies for each of the three tests and the results should be interpreted with caution.

In six studies (Hadlow 2005; Hafner 1998; Krantz 2000; Marchini 2010; Schielen 2006; Wapner 2003), we were able to extract data for the subset of women ≥ 35 years old (≥ 36 years for Schielen 2006). The five NT, PAPP-A, free ßhCG and maternal age test combination studies all showed higher sensitivity and higher FPR for the ≥ 35 years subgroup compared to the < 35 years subgroup as shown on the forest plot (Figure 6) and summary ROC plot (Figure 7). We did not formally compare the two age groups in a meta-analysis because the younger age group had very few cases, thresholds were mixed and there were few studies.

Figure 6. Forest plot of the NT, PAPP-A, free ßhCG and maternal age test strategy by maternal age group (< 35 years versus ≥ 35 years).

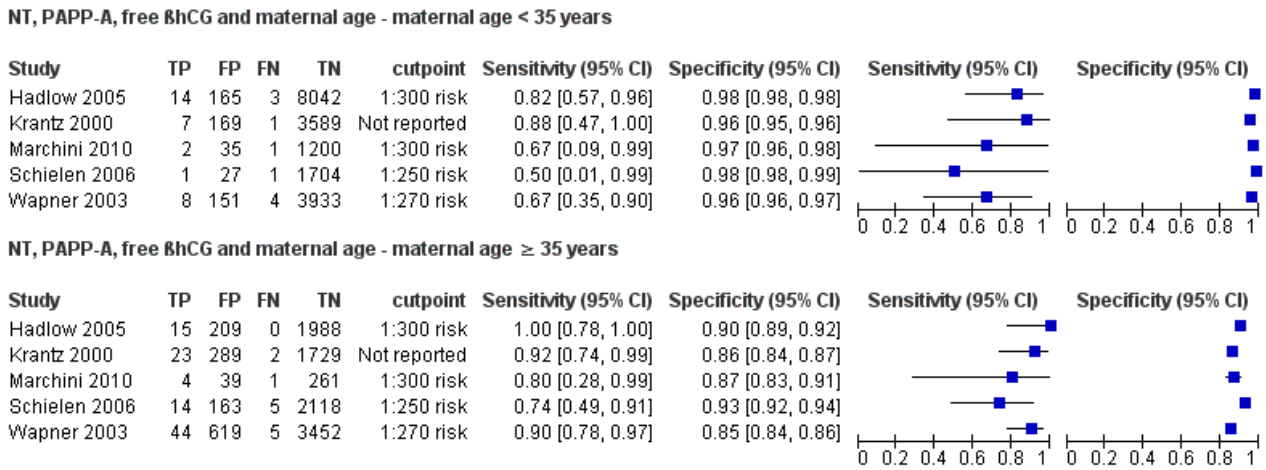
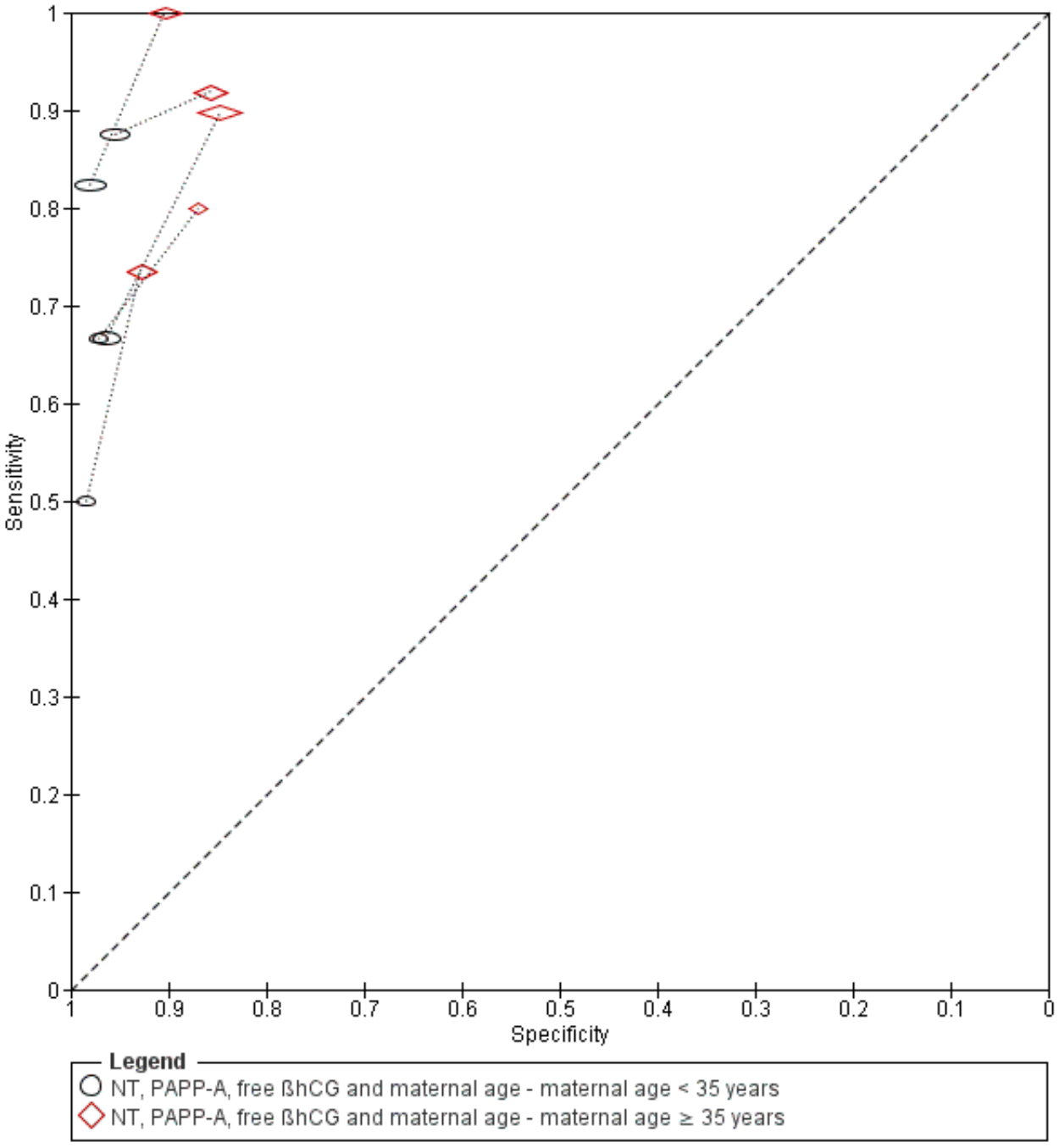


Figure 7. Summary ROC plot of the NT, PAPP-A, free ßhCG and maternal age test strategy by maternal age group (< 35 years versus ≥ 35 years).



Women with multifetal pregnancies were included in six studies (Chasen 2003; Hewitt 1996; Leung 2009; Marchini 2010; Moon 2007; O'Callaghan 2000). Hewitt 1996 evaluated NT alone. Chasen 2003 and O'Callaghan 2000 evaluated the combination of NT and maternal age. Both Leung 2009 and Moon 2007 evaluated nasal bone. Leung 2009 and Marchini 2010 both evaluated the combination of NT, PAPP-A, free βhCG and maternal age. We excluded both studies in a sensitivity analysis to determine the effect on our estimates of test accuracy, due to the potential effect of multifetal pregnancy on serum marker levels. Our findings were unchanged.

DISCUSSION

Summary of main results

We found a large number of studies evaluating first trimester Down's syndrome ultrasound markers with or without first trimester serum screening tests. Few studies compared two or more test strategies in the same population; the majority of studies only evaluated a single test strategy. However, the comparison between NT and the combined NT, PAPP-A, free ßhCG test strategy, both with maternal age, was evaluated in 25 studies. Few studies were available to assess the performance of test strategies involving newer serum markers such as ADAM 12. A summary of results for the 10 most commonly evaluated test strategies is given in Summary of findings 1, and the remaining 50 test strategies are given in Summary of findings 2.

Four key findings were noted.

1. The combined test comprised of NT, PAPP-A, free βhCG and maternal age appears to have significantly better test accuracy than the tests comprised of NT and maternal age with or without either PAPP-A or free βhCG. This combined test detects around nine out of every 10 Down's affected pregnancies for a fixed 5% false positive rate (FPR). By comparison, the tests comprised of NT and maternal age and either PAPP-A or free βhCG, and NT alone or with maternal age detects between seven and eight out of every 10 Down's affected pregnancies for a fixed 5% FPR.
2. While the test combinations that include nasal bone showed good detection rates when combined with PAPP-A and free βhCG, the evidence was limited (three studies) and the variation in threshold precluded meta-analysis.
3. The evidence for combining NT with higher numbers of serum markers showed similar detection rates to combinations of NT and double or triple serum markers that include PAPP-A, but were based on data from only one or two studies. Therefore further evaluation of these tests is needed. Furthermore, there were combinations of NT and other ultrasound markers with serum markers that showed superior detection rates to combinations of NT with standard double markers commonly used in clinical practice, which may warrant further study.
4. Detection rates were lower in high-risk pregnancies (mainly due to advanced maternal age) compared to routine screening populations. Evidence was available for three tests at a fixed 5% FPR and showed reductions in detection rates of between 5% and 25%. Part of this effect may be explained by studies in routine screening populations missing false negative cases lost through increased miscarriage in Down's pregnancies, but this does not fully explain the effect. We were unable to draw any conclusions as to why this may be the case, especially since the analyses were based on few high-risk referral studies. This finding also contradicts the observation we made in five studies where data were available to compare the performance of the NT, PAPP-A, free βhCG and maternal age test strategy between women younger than 35 years and those 35 years or more within the same study. In these studies, the ≥ 35 years age group showed higher detection rates and FPRs compared to the group less than 35 years old. It should be noted that very few cases contributed to the analysis of the younger age group.

Strengths and weaknesses of the review

This review is the first comprehensive review of first trimester ultrasound and serum screening. We examined papers from around the world (32 countries), 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. This is less of an issue for first trimester serum screening, compared to second trimester serum screening, as the majority of authors chose a cut-point of 5% FPR.
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 that are inherently different.
5. We were unable to perform all 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 substantial 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 serum screening plays a major role, usually in combination with first trimester ultrasound scanning. In others 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 first trimester test comprised of nuchal translucency (NT), pregnancy-associated plasma protein A (PAPP-A), free beta human chorionic gonadotrophin (βhCG) and maternal age; there is little evidence to recommend the use of first trimester ultrasound markers alone, combinations with single serum tests or those that exclude PAPP-A. However, the data available on the addition of more that more than two serum markers to ultrasound markers are limited, and based on generally small populations of women. We would not recommend that these tests be introduced into wider clinical practice without careful consideration of cost.

The review has shown that tests involving NT and two or three markers in combination with maternal age are significantly better than those involving ultrasound markers alone. We would therefore recommend that ultrasound markers alone, or combinations involving a single serum marker are not used for Down's syndrome screening. The choice of multiple serum markers will depend on the availability of certain assays in local laboratories. On the basis of this review we would recommend the combination of NT, PAPP-A, free βhCG and maternal age, as it significantly outperforms NT and maternal age or NT and maternal age with either of the two serum markers, and is widely available. The data for other test combinations limits our ability to make any other recommendations about specific test combinations. Alternative screening methods should also be considered when making policy decisions, and are the subject of other reviews in this suite.

Implications for research

Further evaluation of test combinations involving ultrasound markers with three or more serum markers are required to determine whether they offer superior test performance. Further study of the performance of test combinations in women over 35 is required, as this age group has the highest incidence of Down's syndrome and has the greatest requirement for tests with high detection rates.

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]*

Acacio 2001

Clinical features and settings	High-risk referral for invasive testing
Participants	230 participants Brazil - private centres Dates not specified Pregnant women Mean age 35.8 years (21-45 years) Singleton pregnancies Karyotyping performed at same time as NT 10-14 weeks' gestation
Study design	Diagnostic validation study to determine the best ROC cut-off for NT Retrospective study of patient notes
Target condition and reference standard(s)	Down's syndrome: 12 cases Reference standards: chorionic villus biopsy, amniocentesis or blood or placenta used for fetal karyotyping
Index and comparator tests	NT with cut-off of 2.5 mm (found to be optimum cut-off from ROC) (Sequoia, Aspen 128XP10-Acuson and Toshiba SH140)
Follow-up	100% karyotyping
Aim of study	To define the best fixed cut-off point for NT, and the accuracy of this cut-off for all fetal aneuploidy screening and for trisomy of chromosome 21
Notes	

Acacio 2001 *(Continued)*

Table of Methodological Quality

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
Partial verification avoided? All tests	Yes	All patients received a reference standard
Differential verification avoided? All tests	No	Women had different reference standard
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

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), 86% < 35, 14% ≥ 35 Singleton pregnancies

Audibert 2001 *(Continued)*

	10-14 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 karyotyping conducted (in 7.6% of patients) depending on presence of risk > 125, high maternal age, parental anxiety, history of chromosomal defects or parental translocation or abnormal second trimester scan age Cytogenetic testing of newborns with suspected abnormalities Postmortem on terminations of pregnancy or miscarriages Follow-up to neonatal examination in newborn
Index and comparator tests	Maternal age First trimester NT planned at 12-13 weeks, 3 mm risk cut-off Second trimester serum hCG between 14 and 17 weeks (Amerlite, Orthoclinical diagnostics machine), cut-off 1:250 (Prenata software) Second trimester serum AFP between 14 and 17 weeks (Amerlite, Orthoclinical diagnostics machine), cut-off 1:250 (Prenata software) Serum tests in 3790 women
Follow-up	Delivery and postnatal paediatric examination 35 lost to follow-up and excluded from analysis Pregnancy loss in 37 women due to spontaneous abortion (n = 21) or intrauterine death (n = 16) 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.
Notes	Women lost to follow-up are excluded in the final analysis. All antenatally detected cases were terminated.

Table of Methodological Quality

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?	No	Choice of reference standard depended on index test results

Audibert 2001 *(Continued)*

All tests		
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 measured or not recorded in 219 women and these patients were excluded from the study
Withdrawals explained? All tests	Yes	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	3188 participants Cambridge, UK - Maternity Hospital August 2001-March 2004 Singleton pregnancies Pregnant women Median age 37 years (19-46 years) 11-14 weeks' gestation 45-84 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 $\geq$ 3 mm or risk > 1:250 as defined by combined NT and serum results (chorionic villus sampling 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)

Babbur 2005 *(Continued)*

	Second trimester serum biochemistry (AutoDELFIA(TM) time-resolved fluorimmunoassay (Perkin Elmer)) at 14 weeks. Offered to patients with negative first trimester NT (2725 accepted, 85%)
Follow-up	Details of follow-up to birth not given
Aim of study	To determine the detection and false positive rates for trisomy 21 using 2-stage combined nuchal translucency and triple testing whilst disclosing abnormal NT measurements at the scan
Notes	Women with miscarriages excluded

Table of Methodological Quality

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	Yes	463 patients having NT did not go on to have serum testing

Barrett 2008

Clinical features and settings	Routine screening
Participants	10,273 participants with complete screening and outcome data

Barrett 2008 *(Continued)*

	Australia - screening programme, independent ultrasound practices 24-month period (dates not specified) Pregnant women Mean maternal age 34.9 years (screen positive) and 30.5 years (screen negative) Singleton pregnancies 11-13 weeks' gestation
Study design	Cohort study
Target condition and reference standard(s)	Down's syndrome: 32 cases Reference standard: karyotyping or follow-up to birth
Index and comparator tests	NT (FMF protocol) First trimester PAPP-A and free ßhCG (90.2% by time resolved amplified cryptate emission technology, Kryptor random access immunoassay analyzer, Brahms, 9.8% by manual Ortho Clinical Diagnostic Immunometric I^{125} immunoassay for PAPP-A, and Ortho Clinical Diagnostics Vitros ECi automated analyzer for ßhCG) Risk cut-off 1:300
Follow-up	Linkage to data collected by the Midwives Notification System and the Western Australia Birth Defects Registry and by searching laboratory records of all prenatal cytogenetics services in the state. 162 women lost to follow-up were excluded Pregnancy loss in 54 women due to miscarriage (n = 35), stillbirth (n = 17) and neonatal death (n = 2)
Aim of study	To investigate associations between combined first-trimester screen result, pregnancy associated plasma protein level and adverse fetal outcomes in women
Notes	

Table of Methodological Quality

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

Barrett 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

Belics 2011

Clinical features and settings	High-risk referral for invasive testing
Participants	2032 participants with adequate imaging on ultrasound screening Budapest - single centre January 2003 - February 2010 Pregnant women Mean age 36.4 years (15-46 years) (Down's syndrome) and 29.8 years (15-49 years) (no Down's syndrome) 11-20 weeks' gestation
Study design	Cohort study
Target condition and reference standard(s)	Down's syndrome: 52 cases Reference standards: amniocentesis or CVS (85% of women), or follow-up to birth
Index and comparator tests	First and second trimester fetal iliac angle (GE Medical System Kretztechnik GmbH & Co OHG, AC2-5 transabdominal and IC5-9 transvaginal curved array transducer and Medison Co., LTD EC4-9ES transvaginal and C3-7IM transabdominal curved array transducer) Measurement taken from a transverse section of the fetal pelvis Cut-off angles of 75-100º
Follow-up	Followed up to delivery (no cases were detected at birth)
Aim of study	To present results of the sonographic measurement of the fetal iliac angle during the first and second trimesters of pregnancy
Notes	

Belics 2011 *(Continued)*

Table of Methodological Quality

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	Yes	All women received a reference standard
Differential verification avoided? All tests	No	Different reference standards used
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	95.2% had adequate imaging
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 Singleton pregnancies Pregnant women Mean age 32 years (16-46 years), 8.3% > 35 years

Benattar 1999 *(Continued)*

	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), risk cut-point 1:250 First trimester (12-14 weeks) serum AFP and free ßhCG (Elsa AFP and Elsa free ßhCG; 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 are not stated. Unclear whether women were followed up to birth. Of the 1656 women, 12 (0.7%) were lost to follow-up, 2 had miscarriages, 2 had preterm premature ruptures of the membranes and 2 had intrauterine deaths.
Aim of study	To evaluate the sequential combination of ultrasound screening for fetal aneoploidy at 11-14 weeks with maternal biochemistry at 12-14 and 15-18 weeks of gestation
Notes	

Table of Methodological Quality

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?	Yes	Index test interpreted without knowledge of reference standard results

Benattar 1999 *(Continued)*

All tests		
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 London - 2 antenatal clinics January 2003 - December 2008 Pregnant women Median 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	First trimester NT, PAPP-A and free ßhCG (details not reported) Second trimester AFP, uE3, free ßhCG and inhibin A (details not reported) Results in multiple publications
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 standard deviation of NT measurements has decreased over time and, if so, to revise the estimate and assess the effect of revising the estimate of the standard deviation on the performance of antenatal screening for Down's syndrome
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population

Bestwick 2010 *(Continued)*

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

Biagiotti 1998

Clinical features and settings	High-risk referral for invasive testing
Participants	232 participants (all had NT and serum testing) 32 cases of Down's and 200 randomly selected controls (selected from series of 3731 women) Italy - single centre July 1993 - December 1996 Pregnant women 10 to 13 weeks' gestation
Study design	Case-control study
Target condition and reference standard(s)	Down's syndrome: 32 cases Reference standards: CVS or amniocentesis

Biagiotti 1998 *(Continued)*

Index and comparator tests	Maternal age First trimester NT (in longitudinal section of the fetus with caliper measurements to the nearest 0.1 mm) First trimester PAPP-A (Amerlex-M PAPP-A IRMA, Ortho-Clinical Diagnostics) First trimester free ßhCG (Elsa9free ßhCG CIS)
Follow-up	100% karyotyping
Aim of study	To evaluate the potential effectiveness of maternal serum PAPP-A and free ßhCG in combination with NT measurement in the first trimester of pregnancy
Notes	

Table of Methodological Quality

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
Partial verification avoided? All tests	Yes	All women received a reference standard
Differential verification avoided? All tests	Yes	All women had the same reference standard
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

Borenstein 2008

Clinical features and settings	High-risk referral for invasive testing
Participants	516 participants London - hospital birth centre Dates not reported Pregnant women Median maternal age 35 years (range 17-49 years) 11-13 weeks' gestation 16-24 weeks' gestation in a sub-sample of 183 women
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 51 cases Reference standard: CVS
Index and comparator tests	First trimester fetal echocardiography (transabdominally with a 4-8 MHz curvilinear transducer, Voluson 730 Expert, GE Medical Systems) in all women (425 successfully examined) and in the second trimester in 183 women
Follow-up	100% karyotyping
Aim of study	To establish the feasibility of examining the subclavian artery at 11 + 0 to 13 + 6 weeks of gestation and to determine the prevalence of aberrant right subclavian artery (ARSA) in chromosomally normal and abnormal fetuses
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping
Partial verification avoided? All tests	Yes	All women received a reference standard
Differential verification avoided? All tests	Yes	All women received the same reference standard
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded?	No	Reference standard interpreted with knowledge of index test results

Borenstein 2008 *(Continued)*

All tests		
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	425/516 (82.4%) of women were successfully examined
Withdrawals explained? All tests	No	No details of withdrawals given

Borrell 2005

Clinical features and settings	Routine screening
Participants	3731 participants Spain October 1999 - December 2002 Pregnant women 10 to 14 weeks' gestation
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 25 cases Reference standards: CVS (high-risk women) or follow-up to birth
Index and comparator tests	Maternal age First trimester (10-14 weeks) Ductus venous Doppler studies First trimester (10-14 weeks) NT (FMF method) First trimester (10 weeks) serum PAPP-A and free ßhCG (time-resolved fluorescent assays, Perkin-Elmer Life Sciences) Risk cutoffs 1:200, 1:250 or 1:300 DV - Saggital view of quiescent fetus. When optimal record of DV obtained, measured only once. When reversed end diastolic flow present, 3 separated samples obtained. Maximum velocity manually drawn in 3 waveforms and PIV automatically obtained by software linked to equipment
Follow-up	Details given in Borrel 2004: follow-up through phone enquiry, contact with attending obstetrician, births defects registry of Barcelona. Cases with missing follow-up or unknown karyotype excluded from further analysis
Aim of study	To estimate the improvement in screening efficiency when fetal ductus venosus Doppler studies are added to existing first trimester Down's syndrome screening protocols

Borrell 2005 *(Continued)*

Notes

Table of Methodological Quality

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 (described in Borrel 2004)
Partial verification avoided? All tests	Yes	All patients 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	4 unaffected pregnancies could not be assessed with NT
Withdrawals explained? All tests	No	No details of withdrawals given

Borrell 2009

Clinical features and settings	Routine screening and high-risk referral
Participants	7250 participants: 6940 women undergoing routine screening (October 1999 - December 2006) 310 women referred for CVS (October 1999 - December 2007) Barcelona - hospital clinic Pregnant women

Borrell 2009 *(Continued)*

	Mean maternal age 32 years 10-13 and 15-20 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 66 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT and ductus venosus pulsivity index (DVPI) (transabdominal ultrasound, Eccocee SSA and Power-Vision 400, Toshiba Medical Systems, Voluson PRO, General Electrics Healthcare) First trimester PAPP-A and free ßhCG (details not reported) Second trimester AFP, uE3, free ßhCG and inhibin A (details not reported)
Follow-up	From hospital clinic records, telephoning women or from the attending obstetrician. Obtained in 97.4% of pregnancies
Aim of study	To assess the value of ductus venosus blood flow (expressed as pulsatility index, DVPI) in antenatal Down's syndrome screening when used with the combined and integrated tests
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population and 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

Borrell 2009 *(Continued)*

Uninterpretable results reported? All tests	Yes	Ductus venosus measurements were not obtained in 3.3% of pregnancies
Withdrawals explained? All tests	No	No details of withdrawals given

Brameld 2008

Clinical features and settings	Routine screening
Participants	22,280 participants with complete screening results and outcome data August 2001 - October 2003 Australia - State-wide screening programme evaluation Pregnant women Median maternal age 31 years (range 14-47 years), 20% ≥ 35 Singleton pregnancies 10-14 weeks' gestation
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 60 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester PAPP-A, free ßhCG and NT (details not reported) Risk cut-point 1:300
Follow-up	Data on outcome from the Western Australia Midwives data collection, Birth Defects Registry and hospital morbidity and mortality data
Aim of study	To identify first trimester indicators of adverse pregnancy outcomes
Notes	

Table of Methodological Quality

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?	Yes	All women received a reference standard

Brameld 2008 *(Continued)*

All tests		
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

Brizot 2001

Clinical features and settings	Routine screening
Participants	2996 participants Brazil - University Hospital Estimated date of delivery pre December 1999 Pregnant women Median age 28 years (13-46 years), 19.4% ≥ 35 years Singleton pregnancies 10-14 weeks' gestation (mean 12 weeks)
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 10 cases Reference standards: antenatal karyotyping (5.9% of pregnancies: 62% of high-risk, 29% of medium-risk and 3% of the low-risk women) or follow-up to birth (85.3% of women)
Index and comparator tests	Maternal age First trimester (10-14 weeks) NT Risk cut-off 1:300

Brizot 2001 *(Continued)*

Follow-up	85.3% of women were followed up to birth. Of these, 65 were spontaneous miscarriages or intrauterine death with no karyotyping
Aim of study	To assess the detection rate of chromosomal abnormalities using NT
Notes	

Table of Methodological Quality

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

Centini 2005

Clinical features and settings	High-risk patients undergoing routine screening
Participants	408 participants Italy

Centini 2005 *(Continued)*

	Dates not reported Pregnant women Singleton pregnancies Aged ≥ 35 years (range 35-44 years) 10-13 weeks' gestation
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 6 cases Reference standards: amniocentesis in women high risk on screening (16.2%) or follow-up to birth in women who were low risk on screening
Index and comparator tests	Maternal age NT with cut-point 3 mm Serum free ßhCG (Schering RIA) and PAPP-A (Chematil ELISA) Risk score cut-point 1:250
Follow-up	Follow-up at birth in all by collaboration with mothers Women who miscarried were excluded from the study
Aim of study	To evaluate the combined test of NT, serum markers and age in pregnant women 35 years of age and over to detect Down's syndrome
Notes	No live births were Down's syndrome. All detected cases were terminated. 7 women were excluded due to miscarriages

Table of Methodological Quality

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

Centini 2005 *(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	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given

Chasen 2003

Clinical features and settings	Routine screening
Participants	2131 women with 2339 fetuses New York - single centre April 2000 to November 2002 Pregnant women Singleton or multifetal pregnancies Median age 33 years (interquartile range 31-36), 36.2% ≥ 35 years
Study design	Prospective consecutive cohort
Target condition and reference standard(s)	Down's syndrome: 12 cases Reference standards: karyotyping or follow-up to birth in 96.1% of patients
Index and comparator tests	Maternal age NT (FMF methods) Combined risk score cut-point 1:300 Each fetus with a separate chorion was considered individually when calculating the performance of NT but for monochorionic twins, only the fetus with the higher risk calculation was included
Follow-up	Attempted to obtain results for cytogenetic testing following miscarriage or termination or where Down's suspected at birth. Karyotype results or documented evidence of phenotypically normal baby was recorded in 96.1% of patients
Aim of study	To examine the detection rate of chromosomal abnormalities using a combination of nuchal translucency and maternal age
Notes	
Table of Methodological Quality	

Chasen 2003 *(Continued)*

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	Reference standard results were available for only 96% of patients
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	19 patients could not be imaged
Withdrawals explained? All tests	No	No details of withdrawals given

Chen 2009

Clinical features and settings	Routine screening
Participants	242 participants: 22 cases and 220 randomly selected controls China - hospital screening programme August 2003 - March 2007 Pregnant women Median maternal age, cases 30 years (20-44 years) and controls 32 years (19-40 years) 12-14 weeks' gestation
Study design	Case-control study

Chen 2009 *(Continued)*

Target condition and reference standard(s)	Down's syndrome: 22 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	First trimester frontomaxillary facial (FMF) angle (transabdominal ultrasound, ATL HDI 5000, Philips Medical Systems or Voluson 730 Pro, GE Medical systems, by clinicians accredited by the FMF) Measured with a protractor from printed and filed images Angle > 95th percentile taken as positive test result
Follow-up	Pregnancy outcome obtained from obstetric and neonatal files
Aim of study	To evaluate the measurement of FMF angle at 11-13 weeks, 6 days in a Chinese population and its applicability in screening for fetal trisomy 21
Notes	

Table of Methodological Quality

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	Unclear	Unclear if 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	Unclear	Only the most optimal images were included in the study and the proportion of images that were not included is not stated
Withdrawals explained? All tests	No	No details of withdrawals given

Christiansen 2005

Clinical features and settings	Screening programmes for syphilis and Down's syndrome
Participants	108 participants (27 cases of Down's syndrome, 81 controls) Denmark - Statens Serum Institute Dates not specified Pregnant women 5-11 weeks' gestation
Study design	Case-control study
Target condition and reference standard(s)	Down's syndrome: 27 affected cases (18 diagnosed in 2nd trimester, 9 at birth) Reference standard: karyotyping
Index and comparator tests	Maternal age First trimester (week 11-14) NT Frozen samples tested for: First trimester (week 5-11) inhibin A (dimer assay kit MCA 950KZZ, Serotec) First trimester (week 5-11) ßhCG (available for some samples) First trimester (week 5-11) PAPP-A (available for some samples) (combined PAPP-A and ßhCG TrIFMA assay) Risk cutpoints of 1:100, 1:250 and 1:400 Performance assessed with SPlus algorithm
Follow-up	All diagnosis were verified by karyotyping
Aim of study	To investigate whether inhibin A can be used in the first trimester for Down's syndrome screening
Notes	Identified through the Danish central cytogenetic registry as part of quality assurance programme

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping
Partial verification avoided? All tests	Yes	All women had a reference standard
Differential verification avoided?	Yes	All women had the same reference standard

Christiansen 2005 *(Continued)*

All tests		
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	Unclear	Unclear if all index tests 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

Christiansen 2009

Clinical features and settings	Routine screening
Participants	335 participants: 74 cases and 261 controls matched for length of sample storage and maternal age Denmark - screening programme Dates not reported Pregnant women Singleton pregnancies Median maternal age cases 37.5 years and controls 36.4 years 8-13 weeks' gestation
Study design	Case-control study
Target condition and reference standard(s)	Down's syndrome: 74 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (details not reported) Fresh serum samples tested for: First trimester PAPP-A and free ßhCG (AutoDelfia, PerkinElmer, Turku or Kryptor, Brahms) Frozen serum samples tested for:

Christiansen 2009 *(Continued)*

	First trimester placental growth hormone (double monoclonal ELISA, DSL-10-19 200, Diagnostic Systems Laboratory Inc) Growth hormone binding protein (enzyme-amplified ELISA, DSL-10-48 100, Diagnostic Systems Laboratory Inc)
Follow-up	Cross-referencing with the Danish Cytogenetic Central Registry
Aim of study	To examine the potential of placental growth hormone and growth hormone binding protein as maternal serum screening markers for Down's syndrome
Notes	

Table of Methodological Quality

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 some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests 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

Christiansen 2010

Clinical features and settings	Routine screening
Participants	531 participants: 28 cases and 503 controls Denmark - screening programme Dates not specified Pregnant women Singleton pregnancies Median age cases 36 years (range 25-44 years) and controls 29 years (range 17-45 years) 8-14 weeks' gestation
Study design	Case-control study
Target condition and reference standard(s)	Down's syndrome: 28 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 and free ßhCG (details not reported) First trimester ADAM12s (AutoDELFIA/Delfia ADAM12 Research kit 4025-0010, PerkinElmer Life and Analytical Sciences, on the 1235 AutoDELFIA automatic immunoassay system)
Follow-up	Cross-referencing with the Danish Cytogenetic Central Registry
Aim of study	To examine the efficiency of a second generation assay for ADAM12
Notes	

Table of Methodological Quality

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

Christiansen 2010 *(Continued)*

Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests 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

Cicero 2004a

Clinical features and settings	High-risk referral for invasive testing
Participants	970 fetuses (20 twin and 1 triplet pregnancy) UK Dates not specified Pregnant women Median age 37 years (16-48 years) 11-14 weeks' gestation (median 12 weeks)
Study design	Prospective cohort study
Target condition and reference standard(s)	Down's syndrome: 88 cases Reference standard: CVS
Index and comparator tests	Maxillary bone length Mid-saggital view of fetal profile obtained for nasal bone. Transducer angled laterally so that the maxillary bone and mandible including the ramus and condylar process can be seen. Maxillary length measured with callipers. Magnified to 0.1 mm increment
Follow-up	100% karyotyping
Aim of study	To determine the value of measuring maxillary length at 11-14 weeks' gestation in screening for trisomy 21
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description

Cicero 2004a *(Continued)*

Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice
Acceptable reference standard? All tests	Yes	CVS
Partial verification avoided? All tests	Yes	All women had a reference standard
Differential verification avoided? All tests	Yes	All women had the same reference standard
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	Study reports that measurements were made successfully in all cases
Withdrawals explained? All tests	No	No details of withdrawals given

Cicero 2006

Clinical features and settings	Routine screening
Participants	20, 418 participants UK - Fetal Medicine Centre October 2001-2004 Pregnant women Singleton pregnancies Median age 35 years (18-50 years) 11-13 weeks' gestation
Study design	Prospective cohort study

Cicero 2006 *(Continued)*

Target condition and reference standard(s)	Down's syndrome: 140 cases Reference standards: CVS or amniocentesis in high-risk women, or follow-up to birth
Index and comparator tests	Maternal age Presence of nasal Bone (FMF methods) First trimester NT (FMF methods) First trimester serum free ßhCG (Kryptor analyser, Brahms AG) First trimester serum PAPP-A (Kryptor analyser, Brahms AG)
Follow-up	Data on pregnancy outcome from cytogenetics laboratory and by letters and telephone calls to patients, GPs and maternity units 656 patients excluded because karyotype was not known due to miscarriage (n = 185), termination of pregnancy (n = 85) or loss to follow-up (n = 386)
Aim of study	To investigate the impact of incorporating assessment of the nasal bone into first trimester combined screening by fetal nuchal translucency thickness and maternal serum biochemistry
Notes	

Table of Methodological Quality

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?	Yes	Reported that fetal NT and serum markers were successfully measured in all cases

Cicero 2006 *(Continued)*

All tests		
Withdrawals explained? All tests	Yes	Patients lost to follow-up reported

Cocciolone 2008 FTS

Clinical features and settings	Routine screening
Participants	18,901 participants Australia - South Australian Maternal Serum Antenatal Screening Program Dates not reported Pregnant women Median age 31.3 years Maternal and gestational age not reported
Study design	Cohort study
Target condition and reference standard(s)	Down's syndrome: 66 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)
Follow-up	Details not reported
Aim of study	To compare different screening strategies for the detection of Down's syndrome and to consider the practical implications of using multiple screening protocols
Notes	

Table of Methodological Quality

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

Cocciolone 2008 FTS *(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

Cowans 2009

Clinical features and settings	Routine screening
Participants	57,057 participants June 1998 - July 2007 UK - 6 Hospitals Pregnant women Singleton pregnancies Mean age: Down's syndrome 38 years (range 16-49 years) and healthy 29 years (range 13-56 years) 10-14 weeks' gestation
Study design	Cohort study
Target condition and reference standard(s)	Down's syndrome: 723 cases (307 from original cohort and 416 supplemented cases screened at the Fetal Medicine centre or Harris Birthright Research Centre for Fetal Medicine) Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF certified sonographers) First trimester PAPP-A and free ßhCG (Kryptor analyser, Brahms) Rick cut-point 1:300
Follow-up	Birth data collected at birth by the delivering hospital and stored in several databases which were merged. Only women with full records for screening and birth outcome included in the study

Cowans 2009 *(Continued)*

Aim of study	To investigate if fetal sex has an impact on first trimester combined screening for aenuploidy
Notes	

Table of Methodological Quality

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

Cowans 2010

Clinical features and settings	Routine screening
Participants	445 participants: 70 cases and 375 controls matched for storage time and gestational age January 2007 - October 2008 UK Pregnant women

Cowans 2010 *(Continued)*

	Singleton pregnancies Mean maternal age cases 37.0 years (IQR 32.9 to 40.5 years) and controls 32.4 years (IQR 29.0 to 35.9 years) 11-13 weeks' gestation
Study design	Case-control study
Target condition and reference standard(s)	Down's syndrome: 70 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF certified sonographers) Fresh serum samples tested for: First trimester PAPP-A and free ßhCG (Kryptor analyser, Brahms) Frozen serum samples tested for: First trimester placental growth factor (Solid-phase, 2-site fluoroimmunometric research assay (4083-0010) on 6000 DELFIA Xpress random access platform, PerkinElmer)
Follow-up	Karyotype and results for pregnancy outcome received from cytogenetics laboratories and maternity units where deliveries took place
Aim of study	To examine placental growth factor levels in first trimester maternal serum in trisomy 21 pregnancies and to investigate the potential value of PIGF in a first trimester screening test
Notes	

Table of Methodological Quality

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 some index test results

Cowans 2010 *(Continued)*

Index test results blinded? All tests	Unclear	Unclear if all index tests 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

Crossley 2002

Clinical features and settings	Routine screening
Participants	17,229 participants UK - 15 centres Dates not specified Pregnant women Median age 29.9 years, 15.4% ≥ 35 years 10-14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 45 cases Reference standards: CVS (offered where women had high NT measurements), amniocentesis or follow-up to birth
Index and comparator tests	Maternal age NT (FMF method) in 73% of patients Clotted blood samples tested for: Free ßhCG and PAPP-A (Kryptor analyser) in 98.4% of patients
Follow-up	Reported that the outcome of all pregnancies was followed up
Aim of study	To evaluate the use of NT measurement in combination with biochemical markers as a first trimester test for Down's syndrome in routine antenatal setting
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum?	Yes	Routine screening of typical pregnant population

Crossley 2002 *(Continued)*

All tests		
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	Report average success rate of NT (72.9%)
Withdrawals explained? All tests	Yes	Numbers of patients not undergoing NT and biochemical testing given

De Graaf 1999

Clinical features and settings	High-risk referral for invasive testing
Participants	292 participants (207 participants before 14 weeks' gestation) The Netherlands - single centre 19 84-1997 Pregnant women Cases: 37 with Down's syndrome Controls: 255 matched 5:1 with cases for maternal age (within 2 years), gestational age (within 2 weeks) and duration of sample storage (within 2 months) 9-15 weeks' gestation (in a few cases, blood samples for serum testing taken at 15-19 weeks)
Study design	Case-control study

De Graaf 1999 *(Continued)*

Target condition and reference standard(s)	Down's syndrome: 37 cases (24 affected pregnancies in women with NT testing enrolled before 14 weeks' gestation) Reference standards: CVS and amniocentesis
Index and comparator tests	Maternal age NT (FMF methods) with cut-off > 3 mm Frozen serum samples tested for: First trimester free ßhCG and AFP (DELFIA dual labelled time resolved fluorescent assay) First trimester serum PAPP-A (DELFIA research assay (CR61-105)) First trimester serum AFP
Follow-up	100% karyotyping
Aim of study	To determine the expected detection rate and false positive rate for Down's syndrome achievable by early pregnancy screening with combined measurements of serum PAPP-A, free ßhCG and fetal nuchal translucency, with the addition of AFP
Notes	

Table of Methodological Quality

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
Partial verification avoided? All tests	Yes	All women had a reference standard
Differential verification avoided? All tests	Yes	All women had karyotyping
Incorporation avoided? All tests	Yes	Index test did not form part of the reference standard
Reference standard results blinded? All tests	No	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if 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?	Yes	In 11 controls, failed to measure NT

De Graaf 1999 *(Continued)*

All tests		
Withdrawals explained? All tests	No	No details of withdrawals given

Ekelund 2008

Clinical features and settings	Routine screening
Participants	95,645 participants (40,815 in 2005 and 54,830 in 2006) Denmark - 19 obstetrics and gynaecology departments January 2005 - December 2006 Pregnant women Maternal and gestational age not reported First trimester
Study design	Cohort
Target condition and reference standard(s)	Down's syndrome: 225 cases (121 in 2005 and 104 in 2006) Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (by nurses, midwives and doctors in accordance with FMF guidelines) First trimester PAPP-A and free ßhCG (Brahms Kryptor, Brahms Immunodiagnostic Systems or Delfia Xpress, PerkinElmer) Risk cut-point 1:300
Follow-up	Information obtained from the Danish central cytogenetic registry. No details of follow-up for women without pre or post-natal chromosome analysis
Aim of study	To evaluate the impact of a screening strategy in the first trimester, introduced in Denmark during 2004 to 2006, on the number of infants born with Down's syndrome and the number of CVS and amniocentesis, and to determine detection and false positive rates in the screened population in 2005 and 2006
Notes	

Table of Methodological Quality

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

Ekelund 2008 *(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	Information given on the proportion of women not undergoing screening

Gasiorek-Wiens 2001

Clinical features and settings	Routine screening
Participants	21,959 participants Germany, Switzerland and Austria - multicentre study June 1995-May 2000 Pregnant women Median age 33 years (15-49 years), 36.1% > 35 years Singleton pregnancies 10-14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 210 cases Reference standards: CVS, amniocentesis or follow-up to birth
Index and comparator tests	Maternal age NT (FMF methods)

Gasiorek-Wiens 2001 *(Continued)*

	Risk cut-points of 1:100 and 1:300
Follow-up	Follow-up in 92.2% of women. Loss to follow-up was due to miscarriage (n = 258), termination of pregnancy (n = 125) or absence of antenatal karyotyping (n = 1463). Only those with follow-up information included in the study
Aim of study	To examine the effectiveness of screening for Down's syndrome using age and NT at 10-14 weeks of gestation
Notes	

Table of Methodological Quality

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	Reported that NT successfully measured in all cases
Withdrawals explained? All tests	No	No details of withdrawals given

Gasiorek-Wiens 2010

Clinical features and settings	Routine screening

Gasiorek-Wiens 2010 *(Continued)*

Participants	4097 participants with complete data on pregnancy outcome Germany - single examiner December 1997 - November 2006 Pregnant women Singleton pregnancies Median age 35.1 years (range 13.2-46.7 years) 11-13 weeks' gestation
Study design	Cohort study
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 NT (FMF methods) Mixture model, Delta NT and multiple of the median methods
Follow-up	Patient history and ultrasound results were entered into a database and pregnancy outcome or chromosomal results added as they became available 74 (1.8%) of women were excluded from the study because of incomplete follow-up information
Aim of study	To validate the mixture model in a single operator dataset and to compare the detection rates for fetal chromosomal defects obtained from the mixture model with those obtained from either the delta NT or log multiple of the median approach
Notes	

Table of Methodological Quality

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?	No	Reference standard interpreted with knowledge of index test results

Gasiorek-Wiens 2010 *(Continued)*

All tests		
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

Go 2005

Clinical features and settings	Routine screening
Participants	1759 participants The Netherlands - private practice (VU medical centre) May 2001-October 2003 Pregnant women 49% ≤ 35 years, 51% ≥ 36 years 9-14 weeks' gestation
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 21 cases Reference standards: Invasive testing or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF methods using own medians) First trimester PAPP-A and free ßhCG (ELIPS Perkin Elmer, Finland)
Follow-up	Follow-up data from medical records and patient reports. Data from 242 patients (12%) were not available and these patients were excluded from the study.
Aim of study	To determine the diagnostic value of the combination screening test for Down's syndrome in the first trimester of pregnancy
Notes	Dutch language

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum?	Yes	Routine screening of typical pregnant population

Go 2005 *(Continued)*

All tests		
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	Unclear	Unclear if all index tests 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	Incomplete investigation reported in 25 patients (1.2%)
Withdrawals explained? All tests	No	No details of withdrawals given

Gyselaers 2005

Clinical features and settings	Routine screening
Participants	13,267 participants (13,207 participant received both NT test and serum testing) Belgium - multicentre study (35 centres) Data from January 2004-April 2004 added to previous database from before 2003 Pregnant women First and second trimester testing
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 26 cases Reference standards: CVS, amniocentesis or follow-up to birth
Index and comparator tests	Maternal age

Gyselaers 2005 *(Continued)*

	First trimester NT (FMF methods) First trimester PAPP-A (ELISA 2397, DRG International Inc) and free ßhCG (IRMA K1P1001) Second trimester PAPP-A and free ßhCG Risk cut-points of 1:200 and 1:300
Follow-up	Follow-up to birth reported by mail by obstetricians. Non-responding obstetricians contacted personally to obtain missing data. Results of follow-up reported by mail by obstetricians. Non-responding obstetricians contacted personally to obtain missing data Cases of miscarriages (n = 49) and other fetal chromosomal abnormalities excluded from the study. Unclear if other patients lost to follow-up
Aim of study	To evaluate the performance of a first trimester fetal aneuploidy screening programme
Notes	Women with miscarriages or cases of other chromosomal defects were excluded from the study. 9 live births of babies with Down's syndrome

Table of Methodological Quality

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	Yes	Numbers of women excluded due to miscarriage or other chromosomal defects and numbers not undergoing NT and biochemical testing reported.

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 study
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 fluorimmunoassay, 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
Notes	

Table of Methodological Quality

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?	No	Choice of reference standard depended on index test results

Habayeb 2010 *(Continued)*

All tests		
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

Hadlow 2005

Clinical features and settings	Routine screening
Participants	10,436 participants receiving both NT and serum testing and with complete follow-up data Australia Data from 2-year period (dates not specified) Pregnant women Mean age 30.7 years, 21.2% ≥ 35 years Singleton pregnancies 11-14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 32 cases Reference standards: CVS, amniocentesis or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF methods) Clotted blood samples tested for: First trimester PAPP-A (Kryptor random access immunoassay analyser or manual Ortho Clinical Diagnostics Immunometric I125 immunoassay) First trimester free ßhCG (Kryptor random access immunoassay analyser or Ortho Clinical Diagnostics Vitros ECi automated analyser)

Hadlow 2005 *(Continued)*

	Risk cut-point 1:300
Follow-up	Data obtained from WA Midwives notification system and WA Birth defects registry. Missing information sought from referring doctor and ultrasound practice. Data linkage achieved in 10,436 (99.6%) of patients In index test negative patients, outcome for 160 women not known In index test positive patients, outcome in 2 women not known
Aim of study	To audit the initial 2 years of conduct of the combined first trimester screening
Notes	Women with miscarriages or multiple pregnancies were excluded from the study

Table of Methodological Quality

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

Hafner 1998

Clinical features and settings	Routine screening
Participants	4233 participants Austria - single hospital June 1993 to July 1996 Pregnant women Median age 28 years (15-49 years), 6.9% ≥ 35 years 10-13 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 7 cases Reference standards: amniocentesis or CVS in patients with previous Down's pregnancy, > 35 years or with a positive biochemical test result. Other women underwent scan at 22 weeks and, if NT > 2.5 mm special examination directed to examination of fetal heart. Follow-up to birth
Index and comparator tests	First trimester NT (cut-off 2.5 mm) NT taken in saggital section. Distance between the end of the echogenic muscles of the c spine and the inner layer of echogenic skin with callipers on the line
Follow-up	No details given of methods of follow-up. 138 women lost to follow-up
Aim of study	To determine the value of NT measurement for the detection of aneuploidies and other malformations in a low-risk population
Notes	It appears that Down's syndrome was only picked up in cases where CVS or amniocentesis had been conducted and it s not clear if patients were followed up to birth

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Unclear	Amniocentesis or anomalies scan at 22 weeks. Unclear if women were also followed 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?	No	Reference standard interpreted with knowledge of index test results

Hafner 1998 *(Continued)*

All tests		
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 measurement was not possible in 2% of cases
Withdrawals explained? All tests	No	No details of withdrawals given

Has 2008

Clinical features and settings	Routine screening
Participants	1807 participants with successful scans Turkey September 2003 - December 2005 Pregnant women Singleton pregnancies Median maternal age 28.3 years (range 17-45 years) 11-14 weeks' gestation
Study design	Cohort study
Target condition and reference standard(s)	Down's syndrome: 9 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF methods) First trimester fetal nasal bone (experienced maternal fetal specialists) First trimester PAPP-A and free ßhCG (details not reported) Combined cut-point 1:300
Follow-up	Findings recorded in a computer database. Karyotype results obtained directly from the genetics department. Pregnancy outcomes obtained from hospital records or from parents via telephone interview. 110 women (5%) with terminations, miscarriages or malformations and unknown outcome were excluded from the study
Aim of study	To evaluate the contribution of nasal bone assessment in first trimester Down's syndrome screening
Notes	

Has 2008 *(Continued)*

Table of Methodological Quality

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	Yes	Evaluation of nasal bone was not possible in 9 (0.5%) cases
Withdrawals explained? All tests	No	No details of withdrawals given

Hewitt 1996

Clinical features and settings	High-risk referral for invasive testing
Participants	1306 women with 1317 fetuses (11 sets of twins) Australia - 2 hospitals and 2 private practices September 1993 to September 1994 Pegnant women Singleton or multifetal pregnancies Median age 37 years (21-48 years)

Hewitt 1996 *(Continued)*

	10 to 14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 21 cases Reference standard: CVS
Index and comparator tests	First trimester NT (ATL HDI ESP Diagnostic Ultrasound system), cut-point 3 mm or more
Follow-up	100% karyotyping
Aim of study	To evaluate the accuracy of ultrasound measurement of nuchal thickness in first trimester fetuses for predicting fetal karyotype
Notes	No measurement of NT was recorded in 126 cases (9.6%). All down's syndrome fetuses terminated

Table of Methodological Quality

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	CVS
Partial verification avoided? All tests	Yes	All women received a reference standard
Differential verification avoided? All tests	Yes	All women had the same reference standard
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	No measurement of NT was recorded in 126 cases (9.6%)
Withdrawals explained? All tests	No	No details of withdrawals given

Hormansdorfer 2011

Clinical features and settings	Routine screening
Participants	6508 participants with known fetal outcome Germany - 3 prenatal health centres August 1999 - May 2007 Pregnant women Mean maternal age 31.1 years (16-46 years), 22% ≥ 35 years First trimester
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 40 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF standards) First trimester PAPP-A and free ßhCG (no details given) Different software programmes used with and without modification to exclude the role of maternal age
Follow-up	Methods of follow-up not reported. Stated that only women with known fetal outcome were included in the study
Aim of study	To analyse the impact in test performance of 3 widely used first trimester screening software programs if the maternal age was excluded from their calculation algorithm
Notes	

Table of Methodological Quality

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

Hormansdorfer 2011 *(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

Huang 2010

Clinical features and settings	Routine screening
Participants	7118 participants undergoing combined first trimester screening and a fetal abnormality scan Taiwan - single hospital January 2004 - December 2007 Pregnant women Median maternal age 30 years (range 15-47 years) 8-13 weeks' gestation
Study design	Cohort study
Target condition and reference standard(s)	Down's syndrome: 25 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (11-13 weeks' gestation) (FMF accredited obstetricians) First trimester free ßhCG and PAPP-A (8-12 weeks' gestation) (time resolved amplified cryptate emission, automated Kryptor Analyser, Brahms) Combined cut-point 1:300 Second trimester fetal abnormality scan (18-22 weeks' gestation) for intracardiac echogenic focus (ICEF) (In accordance with the American Institute of Ultrasound in Medicine Practice Guideline)
Follow-up	All neonates examined postnatally and Hospital records reviewed
Aim of study	To determine the relation between intracardiac echogenic focus and trisomy 21 in a population of fetuses previously evaluated by first trimester combined screening

Huang 2010 *(Continued)*

Notes

Table of Methodological Quality

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

Jaques 2007

Clinical features and settings	Routine screening
Participants	16,153 participants Australia - State screening programme February 2000 - June 2002 Pregnant women Mean maternal age 33 years (range 16-51 years), 18.5% ≥ 37 years

Jaques 2007 *(Continued)*

	10-13 weeks' gestation
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 63 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF accredited ultrasonologists) First trimester PAPP-A and free ßhCG (details not reported) First trimester AFP, inhibin A and uE3 added to first trimester results for women who were screened at 13 weeks' gestation (augmented screening, number not reported)
Follow-up	Probabilistic record linkage was used to link health records from the Genetic Health prenatal screening database, Perinatal Data Collection Unit and the Birth Defects Register. Written requests for pregnancy outcome were sent to referring health professionals. Pathology and cytogenetics reports were collected for confirmation of birth defects and/or karyotype 151 women were lost to follow-up and these were excluded in the analysis Of the 16,003 women, pregnancy loss in 71 due to miscarriage (n = 68), stillbirth (n = 1) and neonatal death (n = 2)
Aim of study	To follow up and evaluate the state-wide first trimester combined screening programme for Down's syndrome and trisomy 18 at Genetic Health Services Victoria, Australia
Notes	

Table of Methodological Quality

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?	Yes	Index test interpreted without knowledge of reference standard results

Jaques 2007 *(Continued)*

All tests		
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

Jaques 2010 FTS

Clinical features and settings	Routine screening
Participants	38,584 participants Australia - State screening programme 2003 - 2004 Pregnant women Maternal age ≥ 37 years in 16.3% of women First and second trimester
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 110 cases Reference standards: karyotyping (CVS = 774, amniocentesis =1644) or follow-up to birth
Index and comparator tests	Maternal age First trimester NT, PAPP-A and free ßhCG (n = 38,584) (details not reported)
Follow-up	Probabilistic record linkage was used to link health records from the Prenatal Screening Database, prenatal diagnostic data from cytogenetic laboratories, the Victoria Birth Register (Perinatal Data collection Unit) and the Victoria Birth Defects Register
Aim of study	To map prenatal screening and diagnostic testing pathways in Victorian pregnant women during 2003-2004; measure the impact of prenatal diagnostic testing uptake on the effectiveness of prenatal screening for Down's syndrome; and assess factors influencing uptake of diagnostic testing following screening
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population

Jaques 2010 FTS *(Continued)*

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	Yes	Invalid results obtained for 7.4% of first and 0.1% of second trimester screenings
Withdrawals explained? All tests	Yes	48% of pregnant women in the state did not undergo prenatal testing

Kagan 2010

Clinical features and settings	Routine screening
Participants	56,954 participants with available outcome data UK - multicentre July 1999 - April 2007 Pregnant women Singleton pregnancies Mean maternal age 35.4 years (range 14.1 to 52.2 years) 11-13 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 395 cases Reference standards: karyotyping or follow-up to birth

Kagan 2010 *(Continued)*

Index and comparator tests	Maternal age First trimester NT First trimester fetal heart rate (pulsed-wave Doppler) First trimester nasal bone (FMF certified sonographers) First trimester ductus venous flow (FMF certified sonographers) First trimester flow across tricuspid valve (FMF certified sonographers) First trimester PAPP-A and free ßhCG (Kryptor, Brahms AG or Delfia Express, Perkin Elmer) Multiple publications with different test evaluations
Follow-up	Karyotype results and details of pregnancy outcome added to databases as they became available. Women without complete screening and outcome data (n = 3053, 5.1%) were excluded from the study
Aim of study	To examine the performance of first-trimester screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency thickness, fetal heart rate and maternal serum free ßhCG and PAPP-A Other objectives in related publications
Notes	

Table of Methodological Quality

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

Kagan 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

Kim 2006

Clinical features and settings	Routine screening
Participants	2570 participants with available outcome data Korea - hospital and womens healthcare centre January 2001 to December 2001 Pregnant women Mean age 29.9 years (SD 3.3 years) Singleton pregnancies 10-14 weeks' gestation
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 31 cases Reference standard: amniocentesis or CVS in 419 patients considered high risk (NT > 2.5, aged > 35 years, positive biochemical test result, history of chromosomal abnormality, fetal structural abnormality at ultrasound or other reason). Follow-up to birth
Index and comparator tests	First trimester NT (FMF methods) (HDI 3000, ATL, Bothell, WA, USA) 3 measurements taken, largest one used for risk calculation Cut-off 2.5 mm, 3.0 mm or 95th percentile of each CRL
Follow-up	Pregnancy outcomes ascertained from obstetric and neonatal medical records of live or stillborn babies Only patients with known pregnancy outcome included in the study 8 patients who terminated their pregnancies because of structural abnormalities on ultrasound with no karyotyping results were excluded. Karyotyping was performed in intrauterine fetal death (n = 4) cases
Aim of study	To determine the value of NT with different cut-offs for the detection of chromosomal aberrations
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum?	Yes	Routine screening of typical pregnant population

Kim 2006 *(Continued)*

All tests		
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

Koster 2011

Clinical features and settings	Routine screening
Participants	998 participants: 151 cases and 847 controls matched for gestational age, maternal weight, maternal age and storage time The Netherlands - National institute for Public Health and the Environment 2004 - 2006 Pregnant women Singleton pregnancies Median maternal age 37 years (interquartile range 36-39 years) 8-13 weeks' gestation
Study design	Case-control study

Koster 2011 *(Continued)*

Target condition and reference standard(s)	Down's syndrome: 151 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT Fresh serum samples tested for: First trimester free ßhCG and PAPP-A (AutoDELFIA, PerkinElmer) Frozen serum samples tested for: First trimester ADAM 12s, total hCG, placental protein 13 (PP13) and placental growth factor (PlGF) (AutoDELFIA or DelfiaXpress, PerkinElmer)
Follow-up	Pregnancy outcome was recorded via questionnaires and self-reporting by the participating women. Only samples for pregnancies with known outcome were selected as controls
Aim of study	To evaluate the modelled predictive value of 3 current screening markers (PAPP-A, free ßhCG and NT) and 4 potential screening markers (ADAM 12, total hCG, PP13 and PlGF) for Down's syndrome using different screening strategies
Notes	

Table of Methodological Quality

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 some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice

Koster 2011 *(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

Kozlowski 2007 GC

Clinical features and settings	Routine referral
Participants	6906 participants with complete outcome data Germany - gynaecologists practices January 2000 - December 2003 Pregnant women Median maternal age 32 years (15-48 years), 26.4% ≥ 35 years 11-14 weeks' gestation
Study design	Cohort study
Target condition and reference standard(s)	Down's syndrome: 19 cases in gynaecologists practices Reference standard: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF certified gynaecologists) First trimester free ßhCG and PAPP-A (Kryptor analyser, Brahms) Risk cut-point 1:300
Follow-up	Data on pregnancy outcome were obtained by contacting the patient or their general gynaecologist. Women without complete outcome data (36%) were excluded from the study
Aim of study	To evaluate and compare the screening performance for fetal trisomy 21 in the first trimester of pregnancy in general gynaecologists practices and specialised centres for prenatal care in Germany
Notes	

Table of Methodological Quality

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

Kozlowski 2007 GC *(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	Yes	146 women (including 11 with down's syndrome) excluded as results could not be assigned to gynaecologists' or prenatal centre group

Kozlowski 2007 PC

Clinical features and settings	Routine referral
Participants	3862 participants with complete outcome data Germany - tertiary level prenatal centres January 2000 - December 2003 Pregnant women Median maternal age 34 years (range 14-46 years), 43.2% ≥ 35 years 11-14 weeks' gestation
Study design	Cohort study
Target condition and reference standard(s)	Down's syndrome: 26 cases Reference standard: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF certified sonographers) First trimester free ßhCG and PAPP-A (Kryptor analyser, Brahms)

Kozlowski 2007 PC *(Continued)*

	Risk cut-point 1:300
Follow-up	Data on pregnancy outcome were obtained by contacting the patient or their general gynaecologist. Women without complete outcome data (8%) were excluded from the study
Aim of study	To evaluate and compare the screening performance for fetal trisomy 21 in the first trimester of pregnancy in general gynaecologists practices and specialised centres for prenatal care in Germany
Notes	

Table of Methodological Quality

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	Yes	146 women (including 11 with down's syndrome) excluded as results could not be assigned to gynaecologists' or prenatal centre group

Krantz 2000

Clinical features and settings	Routine screening
Participants	10,251 participants

Krantz 2000 *(Continued)*

	USA September 1995 to June 1998 Pregnant women 34.7% ≥ 35 years Singleton pregnancies No diabetes 9-13 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 50 cases (33 had undergone biochemical testing) Reference standards: not reported
Index and comparator tests	Maternal age First trimester NT in 5,809 (2018 ≥ 35 years).patients (FMF methods) Dried blood samples tested for: First trimester free ßhCG and PAPP-A in 10,251 patients (enzyme-linked immunosorbent asay procedures)
Follow-up	No details of follow-up reported
Aim of study	To assess the effectiveness of free ßhCG, PAPP-A and NT for first trimester screening for Down's syndrome and trisomy 18
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Unclear	Unclear reference standard
Partial verification avoided? All tests	Unclear	Unclear if all patients had a reference standard
Differential verification avoided? All tests	Unclear	Unclear if choice of reference depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded?	No	Reference standard interpreted with knowledge of index test results

Krantz 2000 *(Continued)*

All tests		
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

Kublickas 2009

Clinical features and settings	Routine screening
Participants	3907 participants Sweden 2005 - 2006 Pregnant women 51% of women aged ≥ 35 years 9-14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 29 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF trained sonographers) First trimester free ßhCG and PAPP-A (AutoDELFIA, PerkinElmer)
Follow-up	The dataset used contained outcomes for all pregnancies
Aim of study	To provide the necessary mathematical formulae to construct a risk calculation package for Down's syndrome using maternal serum free ßhCG, PAPP-A and NT measurements in the first trimester for use in a web-based system
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum?	Yes	Routine screening of typical pregnant population

Kublickas 2009 *(Continued)*

All tests		
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

Kuc 2010

Clinical features and settings	Routine screening
Participants	27,291 participants: 223 cases and 22,157 controls (not matched) The Netherlands - The Dutch National Institute for Public Health and the Environment Dates not specified Pregnant women Maternal age not reported 8-13 weeks' gestation
Study design	Case-control study
Target condition and reference standard(s)	Down's syndrome: 223 cases Reference standards: karyotyping or follow-up to birth

Kuc 2010 *(Continued)*

Index and comparator tests	Maternal age First trimester NT (FMF trained sonographers) First trimester free ßhCG and PAPP-A (automated dissociation-enhanced lanthanide fluorescent immunoassay, AutoDELFIA, PerkinElmer)
Follow-up	Known outcomes for cases and controls
Aim of study	To estimate the effect of timing of serum collection on screening performance
Notes	

Table of Methodological Quality

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 Hong Kong - multicentre study 1997 to 2000 Pregnant women Mean age 30.5 years (19% ≥ 35 years) (unaffected pregnancies) 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 stated) (All women underwent both NT and biochemical testing)
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 only 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
Notes	

Table of Methodological Quality

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 (6.1% had no ascertainment of pregnancy outcome)
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	No	No details of withdrawals given

Leung 2009

Clinical features and settings	Routine screening
Participants	10,185 participants (178 twin pregnancies; 10,363 fetuses) Hong Kong - University Hospital June 2003 - March 2007 Pregnant women Singleton or multifetal pregnancies Median maternal age 32 years (IQR 30-35 years), 27.4% of women aged ≥ 35 years 11-13 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 34 cases Reference standards: amniocentesis or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF accredited doctors, HDI 5000 or HDI 3000, Philips Medical System) First trimester nasal bone assessment (7925 women) (FMF accredited doctors, HDI 5000 or HDI 3000, Philips Medical System) First trimester PAPP-A and free ßhCG (Kryptor analyser, Brahms Diagnostica GmbH) Risk cut-point 1:300

Leung 2009 *(Continued)*

	For twin pregnancies, a risk was calculated for each fetus based on the individual NT and maternal serum biochemistry corrected for twin pregnancies
Follow-up	Specific staff were allocated to contact all women for pregnancy and fetal outcome. Women were contacted by phone and mail. 5 screen positive and 50 screen negative cases had unknown outcome.
Aim of study	To examine the effectiveness of first trimester fetal trisomy 21 screening using a combination of maternal age, NT and maternal serum free ßhCG and PAPP-A levels in a predominantly Chinese population in Hong Kong
Notes	

Table of Methodological Quality

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	Nasal bone status could not be determined in 176 women (2.2%) (2 with Down's syndrome)
Withdrawals explained? All tests	No	No details of withdrawals given

MacRae 2008

Clinical features and settings	Routine screening

MacRae 2008 *(Continued)*

Participants	18,965 pregnancies UK - University Hospital July 1998 - January 2004 Maternal age not reported 10-13 weeks' gestation
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 37 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (trained sonographers) Risk cut-point 1:300
Follow-up	Information on birth outcome from Harris birthright Research Centre database, the North East Regional Cytogenetic Laboratory, the National Down's syndrome register and the Basildon and Thurrock University Hospital database and, in some cases, maternal and paediatric records. For each case, screening results were linked to cytogenetic results/pregnancy outcome
Aim of study	To evaluate NT scans with a view to comparing findings with other research centres
Notes	

Table of Methodological Quality

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	Index tests did not form part of the reference standard
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

MacRae 2008 *(Continued)*

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

Maiz 2007

Clinical features and settings	High-risk referral for invasive testing
Participants	227 participants UK - single centre Pregnant women Singleton pregnancies Median maternal age 35 years (17-49 years) 11-13 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 20 cases Reference standard: CVS
Index and comparator tests	First trimester presence of mitral gap (Doppler flow traces)
Follow-up	100% karyotyping
Aim of study	To investigate the possible association between a particular pulsed Doppler waveform pattern, mitral gap and trisomy 21 at 11 + 0 to 13 + 6 weeks
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Yes	Karyotyping
Partial verification avoided?	Yes	All women received a reference standard

Maiz 2007 *(Continued)*

All tests		
Differential verification avoided? All tests	Yes	Choice of reference standard did not depend on index test results
Incorporation avoided? All tests	Yes	Index tests did not form part of the reference standard
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

Maiz 2009

Clinical features and settings	Routine screening
Participants	19,614 participants with complete screening and outcome data UK - multicentre January 2006 - May 2007 Pregnant women Singleton pregnancies Median maternal age 34.5 years (14.1-50.1 years) 11-13 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 122 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT and fetal heart rate First trimester ductus venous blood flow velocity waveforms (FMF certified sonographers)

Maiz 2009 *(Continued)*

	First trimester PAPP-A and free ßhCG (Delfia Xpress, PerkinElmer)
Follow-up	Karyotype results and details on pregnancy outcome were added to the database as soon as they became available. Women without complete outcome data (5.3%) were excluded from the study
Aim of study	To investigate the performance of first trimester screening for aneuploidies by including assessment of ductus venosus flow in the combined test of maternal age, fetal NT thickness, fetal heart rate and serum free ßhCG and PAPP-A
Notes	

Table of Methodological Quality

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

Malone 2004

Clinical features and settings	Routine screening

Malone 2004 *(Continued)*

Participants	6324 participants USA - multicentre study (15 centres) May 2002 to December 2002 Pregnant women Mean age 30.1 years (16-47 years), 22.1% ≥ 35 years Singleton pregnancies 10-13 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 11 cases Reference standards: karyotyping in 587 (amniocentesis n = 510; neonatal cord blood n = 41; products of conception and autopsy material n = 31), or follow-up to birth
Index and comparator tests	Nasal bone imaging Fetal image in a perfect saggital plane with fetal spine down. Angle of insonation of ultrasound beam with fetal profile close to 45 degrees. Image magnified significantly until 2 echogenic lines are visible in region of fetal nose. Transducer tilted from side to side to distinguish fetal skin from nasal bone. Deeper echogenic line noted to become more echolucent at its distal end
Follow-up	A tracking programme with up to 10 contact options for each patient used for follow-up Follow-up to birth in 6228 patients (98.5%) and adequate nasal bone imaging in 4801 (75.9%)
Aim of study	To evaluate first trimester nasal bone imaging as a screening tool for aneuploidy
Notes	Only 17% of patients who had miscarriage or termination of pregnancy had karyotype information available

Table of Methodological Quality

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 (1.5% had no ascertainment of pregnancy outcome)
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

Malone 2004 *(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	Yes	Nasal bone screening successful in 4801 cases (75.9%)
Withdrawals explained? All tests	No	No details of withdrawals given

Malone 2005

Clinical features and settings	Routine screening
Participants	38,033 participants USA - multicentre study (15 centres) October 1999 to December 2002 Pregnant women Mean maternal age 30.1 years (SD 5.8 years.); 8199 (21.6%) 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 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 hCG, uE3 and inhibin A in 35,236 patients (92.6%) All data 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

Malone 2005 *(Continued)*

	Follow-up to birth in 36,378 patients (97%)
Aim of study	To evaluate first trimester and/or second trimester screening tool for Down's syndrome
Notes	Unclear which types of patients did not have follow-up data. Appears that aborted/miscarried fetuses did not have follow-up

Table of Methodological Quality

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 only 7.1%
Withdrawals explained? All tests	Yes	Details given for patients who did not undergo different index tests

Marchini 2010

Clinical features and settings	Routine screening
Participants	1521 participants (18 twin and 2 triplet pregnancies; 1543 fetuses) Italy Pregnant women

Marchini 2010 *(Continued)*

	Singleton or multifetal pregnancies Median maternal age 31.3 years (range 18-45 years), 19.7% ≥ 35 years 11-14 weeks' gestation
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 8 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF accredited sonographers) First trimester serum free ßhCG and PAPP-A (Kryptor analyser, Brahms) Risk cut-point 1:300
Follow-up	Follow-up obtained by analysis of fetal karyotype, from patient notes and by telephoning patients
Aim of study	To evaluate the performance of the combined test compared to the NT measurement alone, in fetal aneuploidy screening in the general population and in pregnant women aged 35 years and over
Notes	

Table of Methodological Quality

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

Marchini 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

Marsis 2004

Clinical features and settings	Screening of patients ≥ 35 years of age
Participants	262 participants Indonesia - 4 hospitals January 2001 to January 2003 Pregnant women Mean age 37.7 years (35-43 years) Singleton pregnancies 11-13 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 8 cases Reference standards: amniocentesis (unclear in which patients this was conducted) or follow-up to birth
Index and comparator tests	First trimester NT (all patients) with > 3.0 mm cut-off (FMF methods, Apoge 800-ATL, SSD 680-Aloka, Logic alpha 200 GE, Veluson 730 Pro GE) First trimester nasal bone assessment (97 (55%) patients who also had NT)
Follow-up	Follow-up to birth in patients with no nasal bone and NT > 3 mm. Unclear if screen-negative patients had follow-up to birth
Aim of study	Evaluation of a non-invasive method to screen for Down's syndrome at a maternal age of 35 years or more
Notes	No cases of Down's detected that were not picked up in screening tests

Table of Methodological Quality

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

Marsis 2004 *(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	No	No details of withdrawals given

Marsk 2006

Clinical features and settings	Routine screening
Participants	139 participants: 31 cases and 108 controls (3:1 with cases, matched for time of study, geographic location and to be within 5-year age interval) Sweden - data from Swedish Nuchal Translucency Trial Dates not reported Pregnant women Mean age cases 38.5 years (SD 4.0 years) and controls 35.5 years (SD 4.0 years) Singleton pregnancies 8-14 weeks' gestation
Study design	Case-control study
Target condition and reference standard(s)	Down's syndrome: 31 cases Reference standards: not reported
Index and comparator tests	Maternal age First trimester NT (12-14 weeks) (method not specified)

Marsk 2006 *(Continued)*

	Frozen serum samples PAPP-A and free ßhCG in sample taken at 8-14 weeks (Auto Delfia Instrument) Risk cut-points of 1:250 and 1:350 (Lifecycle software used to calculate risk)
Follow-up	No details of methods used to follow women-up
Aim of study	To determine to what extent adding first trimester serum screening to NT would change the detection rate and test positive rate for Down's syndrome
Notes	Part of NUPP trial

Table of Methodological Quality

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	Unclear	Unclear if all index tests 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	Details given for women who did not agree to take part

Matias 1998

Clinical features and settings	High-risk referral for invasive testing
Participants	486 participants UK and Portugal Dates not reported Pregnant women Singleton pregnancies Median age 35 years (17-46 years) 10-14 weeks' gestation
Study design	Prospective cohort study
Target condition and reference standard(s)	Down's syndrome: 38 cases Reference standard: fetal karyotyping. In cases where NT above 95th percentile or abnormal ductus venousus flow, follow-up scan conducted at 14-16 weeks
Index and comparator tests	Maternal age First trimester NT (SSD, Aloka) First trimester ductus venosus flow velocity: measured transabdominally (5-MHz curvilinear probe, Ecocee, Toshiba) or transvaginally (SSD 2000, Aloka)
Follow-up	100% karyotyping
Aim of study	To assess the possible role of Doppler ultrasound assessment of ductus venous blood flow in screening for chromosomal abnormalities at 11 to 14 weeks of gestation
Notes	

Table of Methodological Quality

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
Partial verification avoided? All tests	Yes	All women received a reference standard
Differential verification avoided? All tests	Yes	All women had the same reference standard
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test

Matias 1998 *(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	Yes	Reported that measurements made successfully in all cases
Withdrawals explained? All tests	No	No details of withdrawals given

Matias 2001

Clinical features and settings	High-risk referral for invasive testing
Participants	515 participants Portugal Dates not reported Pregnant women Median age 35 years (17-46 years) Singleton pregnancies 11-14 weeks' gestation
Study design	Prospective cohort study
Target condition and reference standard(s)	Down's syndrome: 43 cases Reference standards: fetal karyotyping. In cases where NT above 95th percentile, follow-up scan conducted at 14-16 weeks
Index and comparator tests	Maternal age First trimester NT (SSD, Aloka) First trimester ductus venous Doppler evaluation - ductus venosus flow velocity - abnormal flow is defined as absent or reversed flow of blood in the ductus venosus, normal flow defined as presence. Measurement made by obtaining the right ventral midsaggital plane of the fetal trunk in fetal quiescence. Pulsed Doppler gate placed in distal portion of umbilical sinus. 5 consecutive high-quality waveforms used to measure peak velocity during ventricular systole and diastole, the lowest forward velocity during atrial contraction in late diastole and the pulsatility index. Up to 10 minutes allowed for measurements
Follow-up	All women received karyotyping. Unclear if patients followed up to birth

Matias 2001 *(Continued)*

Aim of study	To review the role of Doppler ultrasound in screening for chromosomal abnormalities at 11 to 14 weeks of gestation
Notes	

Table of Methodological Quality

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
Partial verification avoided? All tests	Yes	All women had a reference standard
Differential verification avoided? All tests	Yes	All women had the same reference standard
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	Reported that Doppler measurements made successfully in all cases
Withdrawals explained? All tests	No	No details of withdrawals given

Mavrides 2002

Clinical features and settings	High-risk referral for invasive testing
Participants	256 participants who were referred to unit for fetal karyotyping and had NT and Doppler studies UK - tertiary referral fetal medicine unit Conducted over 18 months, dates not reported

Mavrides 2002 *(Continued)*

	Pregnant women Median age 35 years (15-42 years) 11-14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 30 cases Reference standard: CVS or follow-up
Index and comparator tests	Maternal age First trimester NT First trimester ductus venous Doppler studies (ATI HDL 5000 US machine with curvilinear TV probe)
Follow-up	Follow-up based on ultrasounds findings, examination at birth, postmortem examination in cases of intrauterine death or termination of pregnancy and by telephone interviews with parents
Aim of study	To assess the role of first trimester Doppler assessment of the ductus venosus in screening for fetal aneuploidy in pregnancies at 11-14 weeks of gestation
Notes	2 live births with Down's syndrome. Appears to be a high-risk invasive testing study but some people did not appear to get karyotyping but were followed up. Probably the majority got karyotyping

Table of Methodological Quality

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
Partial verification avoided? All tests	Yes	All participants had a reference standard
Differential verification avoided? All tests	Yes	All participants had the same reference standard
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

Mavrides 2002 *(Continued)*

Uninterpretable results reported? All tests	Yes	Doppler studies failed in 4 cases (1.5%)
Withdrawals explained? All tests	No	No details of withdrawals given

Maxwell 2011 FTS

Clinical features and settings	Routine screening
Participants	32,478 participants with available outcome data Australia - screening programme 2005 - 2006 Pregnant women Singleton pregnancies Median maternal age 31 years (14-48 years), 24.3% of women aged ≥ 35 years 10-13 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 94 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) Risk cut-point 1:300
Follow-up	Diagnostic data collected from cytogenetic laboratories. Screening data linked to Western Australia diagnostic data, hospital morbidity and mortality data, midwives notification data and the Birth Defects Registry data through the Department of Health Western Australias Data Linkage Branch. Outcome data available for 92.3% of screened women
Aim of study	To investigate socio-demographic characteristics in the uptake of prenatal aneuploidy screening in Western Australia and to identify potential barriers to screening access
Notes	

Table of Methodological Quality

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

Maxwell 2011 FTS *(Continued)*

All tests		
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

Maymon 2005

Clinical features and settings	Routine screening
Participants	595 participants Israel January 1999 - January 2004 Pregnant women Mean age, healthy 30.3 years (SD 4.5), Down's syndrome 33.7 years (SD 4.9) Singleton pregnancies 11-14 weeks' gestation and second trimester screening
Study design	Case-control study
Target condition and reference standard(s)	Down's syndrome: 24 cases Reference standards: amniocentesis (recommended for women with higher risk on first or second trimester testing) or follow-up to birth

Maymon 2005 *(Continued)*

Index and comparator tests	Maternal age First trimester NT (11-14 weeks) First trimester PAPP-A and free ßhCG (methods detailed in Maymon 2001) (some analysed retrospectively from banked samples) Second trimester PAPP-A and free ßhCG (methods detailed in Maymon 2001)
Follow-up	Delivery outcome obtained by telephone interview or medical records. Information was available for all uneventful pregnancies and delivery outcomes. It is unclear whether information on terminations of pregnancy or miscarriages was available.
Aim of study	To evaluate the cross-trimester multiple marker correlation and the minimum marker combination needed for detecting various chromosomal aneuploides
Notes	

Table of Methodological Quality

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

Maymon 2008

Clinical features and settings	Routine screening
Participants	243 participants: 19 cases and 224 consecutive controls USA - antenatal sonographic unit October 2005 - May 2007 Pregnant women Singleton pregnancies 11-13 and 14-28 weeks' gestation
Study design	Case-control study
Target condition and reference standard(s)	Down's syndrome: 19 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (according to FMF criteria) Second trimester nuchal skin-fold (according to published criteria) First trimester free ßhCG and PAPP-A (details not reported)
Follow-up	Cases detected through karyotyping. Stated that controls had normal pregnancies
Aim of study	To assess whether there is a correlation between nuchal translucency and nuchal skin-fold measurements in Down's syndrome and in normal pregnancies
Notes	

Table of Methodological Quality

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	Different reference standards used
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test

Maymon 2008 *(Continued)*

Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests 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

Merz 2011

Clinical features and settings	Routine referral
Participants	124,205 participants Germany Dates not reported Pregnant women Maternal age not reported Singleton pregnancies First trimester
Study design	Retrospective cohort study
Target condition and reference standard(s)	Down's syndrome: 500 cases Reference standard: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (only data obtained by level II or III certified sonographers included) First trimester free ßhCG and PAPP-A (Brahms Kryptor system) FMF Germany risk calculation Risk cut-point 1:150
Follow-up	Details not reported
Aim of study	To demonstrate that the variability of the FPR can be reduced through adjusting the concentrations of free ßhCG and PAPP-A measured in the maternal serum by meaning of a nonlinear regression function modelling the dependence of these variables on maternal weight

Merz 2011 *(Continued)*

Notes

Table of Methodological Quality

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

Michailidis 2001

Clinical features and settings	Routine screening
Participants	7447 participants UK - hospital maternity unit January 1995 to January 2000 Pregnant women Mean age 30.1 years (13-50 years), 21.1% ≥ 35 years, 11.9% ≥ 37 years

Michailidis 2001 *(Continued)*

	10-14 weeks' gestation
Study design	Prospective cohort study
Target condition and reference standard(s)	Down's syndrome: 23 cases Reference standards: karyotyping in women considered at risk due to index test results, age or family history or those with considerable anxiety (632 women, 8.5%). Follow-up to birth
Index and comparator tests	Maternal age First trimester NT in all patients (fetus in mid-sagittal section. Maximum thickness of subcutaneous translucency between skin and soft tissue overlying the C-spine with the fetus in the ventral position) Second trimester AFP, free ßhCG in 65% of patients with NT (radio-immunoassay and immunoradiometric assays)
Follow-up	Outcome at birth assess from hospital database, labour ward records or directly from patients. Follow-up data in 7447 patients (87% of initial patient cohort). Patients without follow-up excluded
Aim of study	To asses the effectiveness of antenatal screening for trisomy 21 by first trimester sonography followed by second trimester biochemical screening
Notes	2nd trimester data not analysed 4 live births: 1 diagnosed before birth and chose not to abort. 3 diagnosed after birth (no invasive testing was conducted)

Table of Methodological Quality

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?	Yes	Information available as would be in standard clinical practice

Michailidis 2001 *(Continued)*

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

Molina 2010 high risk

Clinical features and settings	High-risk referral for invasive testing
Participants	333 participants Spain - fetal medicine unit February 2007 - January 2009 Pregnant women Singleton pregnancies Mean maternal age 32.7 years (range 16.7-47.5 years) 11-14 weeks' gestation
Study design	Cohort
Target condition and reference standard(s)	Down's syndrome: 20 cases Reference standard: CVS
Index and comparator tests	First trimester nasal bone (FMF certified sonographer) First trimester ductus venosus (FMF certified sonographer) First trimester tricuspid regurgitation (FMF certified sonographer)
Follow-up	100% karyotyping
Aim of study	To evaluate detection and false positive rates of the ultrasound markers - nasal bone, ductus venosus flow and tricuspid regurgitation, during the first trimester in a population at high-genetic risk and to study the influence of a 2-stage screening policy after previous combined screening on the rate of invasive procedures
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice
Acceptable reference standard?	Yes	Karyotyping

Molina 2010 high risk *(Continued)*

All tests		
Partial verification avoided? All tests	Yes	All women received a reference standard
Differential verification avoided? All tests	Yes	Choice of reference standard did not depend on index test results
Incorporation avoided? All tests	Yes	Index tests did not form part of the reference standard
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	5 (1.5%) women did not have measurements obtained for nasal bone and tricuspid regurgitation and 10 (3%) did not have measurements obtained for ductus venosus
Withdrawals explained? All tests	No	No details of withdrawals given

Molina 2010 screening

Clinical features and settings	Routine screening
Participants	6831 participants Spain - fetal medicine unit February 2007 - January 2009 Pregnant women Maternal age not reported 9-11 weeks' gestation
Study design	Cohort
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 (FMF certified sonographer)

Molina 2010 screening *(Continued)*

	First trimester PAPP-A and free ßhCG (DELFIA Xpress random access platform, PerkinElmer)
Follow-up	Details not reported
Aim of study	To evaluate detection and false positive rates of the ultrasound markers - nasal bone, ductus venosus flow and tricuspid regurgitation, during the first trimester in a population at high-genetic risk and to study the influence of a 2-stage screening policy after previous combined screening on the rate of invasive procedures
Notes	

Table of Methodological Quality

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	Index tests did not form part of the reference standard
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

Monni 2005

Clinical features and settings	Routine screening

Monni 2005 *(Continued)*

Participants	16,654 participants Italy - single centre 2001-2004 Pregnant women Median age 32 years (14-49 years) Singleton pregnancies 10-14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 96 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF methods, No information given regarding machines used) First trimester nasal bone examination (transabdominal ultrasound in mid-sagittal view) Annual audit of screening performance (medians)
Follow-up	Outcome at birth as recorded in hospital database (provided by outcome sheets or telephone interviews). Of 32,000 cases in the database, 16,654 (52%) patients had NT, nasal bone assessment and follow-up data available. Patients without follow-up data were excluded from the study
Aim of study	To evaluate the feasibility and diagnostic accuracy of fetal NT and nasal bone assessment at 11-14 weeks for screening of trisomy 21
Notes	

Table of Methodological Quality

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?	No	Reference standard interpreted with knowledge of index test results

Monni 2005 *(Continued)*

All tests		
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	In 13 cases (1.3%) not possible to ascertain if nasal bone was visible
Withdrawals explained? All tests	No	No details of withdrawals given

Montalvo 2005

Clinical features and settings	Routine screening
Participants	4538 participants who had follow-up data available Spain - tertiary hospital July 1999 - October 2004 Pregnant women Mean age 31.1 years (14-49 years), 25.9% of patients ≥ 35 years Singleton pregnancies 10-14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 19 cases Reference standards: invasive testing offered to women considered high risk from screening results or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (Methods described by Nicholaides) First trimester PAPP-A and free ßhCG (Kryptor Trace system, CIS Bio International) Risk cut-point 1:270
Follow-up	Only patients with postnatal results available are included in the study
Aim of study	To report the experience of using of use of the combined first trimester screening test
Notes	

Table of Methodological Quality

Montalvo 2005 *(Continued)*

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

Moon 2007

Clinical features and settings	Routine screening
Participants	6471 fetuses with available outcome data Korea July 2004 - March 2006 Pregnant women Singleton or multifetal pregnancies Mean maternal age: Down's syndrome 35.5 years (SD 4.8 years), non-Down's syndrome 31.7 years (SD 3.4 years) 11-14 weeks' gestation

Moon 2007 *(Continued)*

Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 15 cases Reference standard: karyotyping or follow-up to birth
Index and comparator tests	First trimester fetal nasal bone assessment (Voluson 730, LOGIQ 400 or 5, GE Medical Systems or HDI 500, Philips Medical systems) (American Registry of Diagnostic Medical Sonographers certified Sonographers)
Follow-up	Obstetric and neonatal outcome obtained from medical records, karyotyping reports and, when needed, telephone conversations with parents or physicians. A total of 7834 fetuses were included in the study but 1047 fetuses (13.4%) without available outcome data were excluded. The remaining 6787 fetuses included 154 twin pregnancies. Assessment of fetal nasal bone was possible in 6490 (95.6%) of the 6787 fetuses. Comparison of nasal assessments between the control population and Down's cases was performed in 6471 fetuses
Aim of study	To evaluate the role of nasal bone assessment in first-trimester screening for Down's syndrome in the Korean population
Notes	

Table of Methodological Quality

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	Index tests did not form part of the reference standard
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	Assessment of fetal nasal bone was not possible in 297 women (4.4%)

Moon 2007 *(Continued)*

Withdrawals explained? All tests	No	No details of withdrawals given

Muller 2003

Clinical features and settings	Routine screening
Participants	5694 participants who had first trimester NT and biochemical testing France - 9 centres serving 12 maternity units January 1998 - June 2001 Pregnant women Singleton pregnancies Maternal age not reported 11-13 weeks' gestation
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 26 cases Reference standards: invasive testing (offered to women with high NT measurement) or follow-up to birth
Index and comparator tests	Maternal age First trimester nuchal translucency in 98% of patients (methods not specified. 60 sonographers - 2 trained by Fetal Medicine Foundation, who trained 30 in turn. 8 received specific training in France, and 20 were self-taught. Machines not specified) Frozen serum tested for: First trimester PAPP-A (99% of patients), free ßhCG 99% of patients and AFP (93% of patients) (time-resolved fluorescent assay, Perkin-Elmer Life sciences) Risk cut-point 1:250
Follow-up	Data from the French national screening programme used for follow-up at birth. 211 women (3.7%) who did not return after NT or were found to be > 14 weeks were excluded. It is unclear how many patients had follow-up to birth
Aim of study	Prospective study of NT and retrospective evaluation of serum (in same patient population) to evaluate whether or not to move the national French Down's screening programme to a first trimester programme
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population

Muller 2003 *(Continued)*

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	Yes	Women with NT too small to measure assumed to have NT of < 0.5 mm.
Withdrawals explained? All tests	Yes	Women failing to return or who more than 14 weeks pregnant were excluded (214).

Nicolaides 1992

Clinical features and settings	High-risk referral for invasive testing
Participants	827 participants UK - research centre for fetal medicine January 1990 - October 1991 Pregnant women Median age 38 years (22-47 years) 10-14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 13 cases Reference standards: fetal karyotyping by amniocentesis (52%) or CVS (48%)

Nicolaides 1992 *(Continued)*

Index and comparator tests	Maternal age First trimester NT (curvilinear 5MHz transducer, Aloka 650 CO Limited)
Follow-up	100% karyotyping
Aim of study	To examine the significance of fetal NT at 10-14 weeks' gestation in the prediction of abnormal fetal karyotype
Notes	

Table of Methodological Quality

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	Amniocentesis or CVS
Partial verification avoided? All tests	Yes	All women had a reference standard
Differential verification avoided? All tests	No	Women had different reference standards
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

Nicolaides 2005

Clinical features and settings	Routine screening

Nicolaides 2005 *(Continued)*

Participants	75,821 participants with available information on outcome UK - Various hospitals and a fetal medicine centre June 1998 - December 2003 Pregnant women Median age 31 years (13-49 years) Singleton pregnancies 11-13 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 325 cases Reference standards: amniocentesis or CVS (patients considered high risk based on screening). First trimester presence/absence of nasal bone, presence/absence of tricuspid regurgitation or normal/abnormal Doppler studies (patients of intermediate risk on first trimester screening and did not undergo CVS or amniocentesis. With the addition of information from these tests, if adjusted risk was high, CVS was performed). Follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF methods) First trimester free ßhCG and PAPP-A (Kryptor analyser, Brahms AG) Risk cut-point 1:300
Follow-up	Follow-up data from cytogenetics laboratories, patients, GPs or maternity units where they delivered. Patients without follow-up information due to miscarriage or termination (n = 490) or loss to follow-up (n = 2117) were excluded from the study.
Aim of study	To evaluate the performance of first trimester screening for trisomy 21 by a combination of maternal age, fetal NT and maternal serum free ßhCG and PAPP-A. In addition, the impact of a new individual risk orientated 2-stage approach to first trimester screening was examined
Notes	

Table of Methodological Quality

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

Nicolaides 2005 *(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	Yes	Exclusions due to loss to follow-up and missing information for women with miscarriages or terminations of pregnancy explained

Niemimaa 2001

Clinical features and settings	Routine screening
Participants	2515 participants Finland - primary care centres and maternity clinics of hospitals During 1999 Pregnant women 17.5% aged ≥ 35 years 10-13 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 8 cases Reference standards: invasive testing (patients considered high risk based on NT screening) or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (≥ 3 mm) (64% of women) (method not described) Fresh serum tested for: First trimester free ßhCG and PAPP-A (Wallac analytes and 1st trimester risk calculation programme maternal weight correction) Risk cut-point 1:250
Follow-up	Follow-up data from maternity clinics and the National Research and Development Centre for Welfare and Health. Test negative patients followed up by contacting all maternity clinics and the National Re-

Niemimaa 2001 *(Continued)*

	search and Development Centre for Welfare and Health. Unclear if follow-up information was obtained in all cases
Aim of study	To evaluate efficacy of combining first trimester maternal serum and fetal NT measurement in screening for Down's syndrome in Finland
Notes	

Table of Methodological Quality

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

Noble 1995

Clinical features and settings	Routine screening in a high-risk population
Participants	2529 participants UK

Noble 1995 *(Continued)*

	October 1994 to April 1995 Pregnant women Singleton pregnancies Median age 34 years (15-47 years), 47% ≥ 35 years 10-14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 61 cases Reference standards: karyotyping performed (27% of women) due to increased NT (14%), advanced maternal age (10%), previous chromosomally abnormal child (0.5%) or parental anxiety (2%). Ultrasound examination at 20 weeks (65% of patients). Follow-up to birth (9% of women)
Index and comparator tests	Maternal age First trimester NT (methods not stated) Fresh serum (or serum frozen over a weekend) tested for: First trimester free ßhCG (immunoradiometric assay, CIS)
Follow-up	Pregnancy outcome obtained from maternity units or the patients themselves. Follow-up information only appears to have been obtained in 9% of cases (second trimester ultrasound used as reference standard for other women)
Aim of study	To measure the contribution of maternal serum free beta hCG in a screening programme for fetal trisomy 21 based on fetal NT in the first trimester of pregnancy
Notes	No proper results data are presented for this study

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	No	Invasive testing, ultrasound at 20 weeks 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

Noble 1995 *(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	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given

O'Callaghan 2000

Clinical features and settings	Routine screening
Participants	1000 participants Australia - public and private sector venues September 1997 to September 1999 Pregnant women Singleton or multifetal pregnancies (2000 fetuses including 25 sets of dichorionic twins, 7 sets of monochorionic twins and 4 sets of triplets but the numbers amongst the 1000 fetuses reported in the paper were not stated) Median age 32 years 11-14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 8 cases Reference standards: CVS, amniocentesis, neonatal karyotyping or follow-up to birth
Index and comparator tests	Maternal age NT (FMF methods)
Follow-up	Follow-up from cytogenetics laboratory records but the completeness of follow-up is not reported
Aim of study	To evaluate a risk assessment tool based on first trimester NT
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population

O'Callaghan 2000 *(Continued)*

Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Unclear	Unclear if all women had a reference standard
Differential verification avoided? All tests	Unclear	Unclear if the 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

O'Leary 2006

Clinical features and settings	Routine screening
Participants	22,340 participants Australia - 13 ultrasound practices August 2001 to October 2003 Singleton pregnancies Pregnant women Median age 31 years (14-47 years), 20% ≥ 35 years 11-13 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 60 cases

O'Leary 2006 *(Continued)*

	Reference standards: CVS or amniocentesis (women assessed to be high risk on screening) or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF methods) First trimester free ßhCG and PAPP-A (machine not stated) All study participants underwent all tests Risk cut-point 1:300
Follow-up	Follow-up data obtained by review of the Midwives Notification System and the Birth Defects Registry. 415 patients (1.8%) excluded due to no follow-up data. Patients with multiple pregnancies or incomplete screens (n = 3946) were also excluded from the study
Aim of study	To assess fetal outcomes for pregnancies identified at increase risk for Down's syndrome by first trimester combined ultrasound examination and maternal serum biochemistry
Notes	Appears likely that patients with miscarriages and terminations excluded

Table of Methodological Quality

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

O'Leary 2006 *(Continued)*

Withdrawals explained? All tests	Yes	Details given of patients excluded due to incomplete screening data or loss to follow-up

Okun 2008 FTS

Clinical features and settings	Routine screening
Participants	14,487 participants undergoing first trimester screening (a separate cohort of 30,792 pregnancies were evaluated for integrated screening) November 2002 - December 2005 Canada - 2 hospitals Pregnant women Singleton pregnancies Mean maternal age 34 years 11-14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 62 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) Risk cut-point 1:200 or NT ≥ 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
Notes	

Table of Methodological Quality

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

Okun 2008 FTS *(Continued)*

All tests		
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

Orlandi 1997

Clinical features and settings	Routine screening of general and high-risk women
Participants	2010 participants (744 in subgroup undergoing NT testing) Italy Dates not reported Recruited through private physician or genetic counselling program for women of advanced maternal age Pregnant women Aged 15-46 years, 35% ≥ 35 years Singleton pregnancies 9-13 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 11 cases (7 in subgroup with NT testing) Reference standards: not reported

Orlandi 1997 *(Continued)*

Index and comparator tests	Maternal age First trimester NT (37% of patients) (FMF methods, Toshiba SSA 250A or Acuson XP 10) First trimester free ßhCG and PAPP-A (all patients) (dried blood samples, enzyme-linked immunosorbent assays) Risk cut-point 1:380
Follow-up	Not reported
Aim of study	To evaluate first trimester combined screening for Down's syndrome
Notes	Unclear as to what reference standard (if any) was used. All cases of Down's syndrome identified had been picked up by screening

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population
Acceptable reference standard? All tests	Unclear	Reference standard not reported
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard
Differential verification avoided? All tests	Unclear	Unclear if the choice of reference standard depended on screening 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	Details given of women undergoing NT but not biochemical testing

Orlandi 2003

Clinical features and settings	Routine screening (2 centres) or in referred patients (1 centre)
Participants	1089 participants undergoing fetal nasal bone assessment Italy/The Netherlands - 3 centres February 2002 to April 2002 Pregnant women Singleton pregnancies Median age 31.7 years (SD 4.0) in unaffected cases and 36.5 years (SD 4.1) in affected cases 11-14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 15 cases Reference standards: CVS or amniocentesis (women considered high risk on screening on the basis of NT and biochemical results, but not on nasal bone screening, or if requested due to age or anxiety), or follow-up to birth
Index and comparator tests	Maternal age First trimester nasal bone assessment First trimester NT First trimester free ßhCG First trimester PAPP-A
Follow-up	Reported that karyotyping was performed postnatally. It is unclear in which cases this was conducted
Aim of study	To assess the feasibility of measuring nasal bone length in first trimester pregnancy and to confirm if the absence of fetal nasal bone is a marker for Down's syndrome
Notes	

Table of Methodological Quality

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

Orlandi 2003 *(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	Nasal bone assessment was successfully conducted in 94.3% of women
Withdrawals explained? All tests	No	No details of withdrawals given

Orlandi 2005

Clinical features and settings	Routine screening
Participants	2411 participants Italy Dates not reported Pregnant women Median age 30.5 years (SD 8.2) First trimester (gestational weeks not reported)
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 15 cases Reference standard: not reported
Index and comparator tests	Maternal age First trimester nasal bone assessment (FMF methods) First trimester NT, free ßhCG and PAPP-A Data from other studies used to generate statistical parameters to estimate performance of first trimester screening with and without nasal bone evaluation) Risk cut-point 1:250
Follow-up	No details reported for any follow-up to birth

Orlandi 2005 *(Continued)*

Aim of study	To determine the benefit of including nasal bone assessment in addition to standard first trimester markers as a screening test for Down's syndrome
Notes	

Table of Methodological Quality

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	Unclear	Unclear if the choice of reference standard depended on screening results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	Unclear	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

Otaño 2002

Clinical features and settings	High-risk referral for invasive testing
Participants	194 participants Argentina October 2001 - January 2002

Otaño 2002 *(Continued)*

	Pregnant women Median age 36 years (19-44 years) Singleton pregnancies 11-14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 5 cases Reference standard: CVS
Index and comparator tests	First trimester nasal bone assessment (frontal saggital section of the fetal face. Angle of insonation of fetal nose close to 90 degree angle)
Follow-up	100% karyotyping
Aim of study	To evaluate the association of nasal bone on ultrasound and Down's syndrome fetuses at 11-14 weeks' gestation
Notes	States in text that there were 6 cases of trisomy 21

Table of Methodological Quality

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	CVS
Partial verification avoided? All tests	Yes	All women received a reference standard
Differential verification avoided? All tests	Yes	All women had the same reference standard
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test
Reference standard results blinded? All tests	Unclear	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?	Yes	Unsuccessful nasal bone assessment in 6%

Otaño 2002 *(Continued)*

All tests		
Withdrawals explained? All tests	No	No details of withdrawals given

Pajkrt 1998

Clinical features and settings	Routine screening
Participants	1473 participants The Netherlands tertiary maternity unit June 1994 to March 1997 Pregnant women Mean age 31.4 years (SD 5.7), 24% ≥ 35 years Singleton pregnancies 10-14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 9 cases Reference standards: prenatal karyotyping offered to patients considered high risk or maternal anxiety (conducted in 24%) or follow-up to birth
Index and comparator tests	Maternal age NT (FMF method, Hitachi machines, 6 sonographers instructed to take 'sufficient time') Risk cut-point ≥ 3 mm
Follow-up	Follow-up to outcome assessment in the delivery room. 68 women (4.4%) were excluded from the study due to loss to follow-up
Aim of study	To evaluate the effectiveness of NT measurement in the detection of trisomy 21 in a low-risk population
Notes	

Table of Methodological Quality

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?	Yes	All women received a reference standard

Pajkrt 1998 *(Continued)*

All tests		
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	Unclear	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	Unsuccessful NT measurement in 4.3%
Withdrawals explained? All tests	No	No details of withdrawals given

Pajkrt 1998a

Clinical features and settings	High-risk referral for invasive testing
Participants	2247 participants undergoing NT and fetal karyotyping The Netherlands - prenatal diagnostic centre February 1994 to July 1997 Singleton pregnancies Pregnant women Mean age 37.6 years (22-46 years) 10-14 weeks' gestation
Study design	Consecutive cohort
Target condition and reference standard(s)	Down's syndrome: 36 cases Reference standard: prenatal karyotyping
Index and comparator tests	Maternal age FT NT (maximal saggital thickness of NT, corrected for gestational age)
Follow-up	100% karyotyping

Pajkrt 1998a *(Continued)*

Aim of study	To examine the discriminatory capacity of NT measurement in the detection of trisomy 21 and other chromosomal anomalies
Notes	No follow-up information on 12 miscarriages

Table of Methodological Quality

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
Partial verification avoided? All tests	Yes	All women received a reference standard
Differential verification avoided? All tests	Yes	All women had the same reference standard
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	Unsuccessful NT measurement in 2.4%
Withdrawals explained? All tests	Yes	Patients excluded due to sonographically detected fetal abnormalities at NT measurement, no karyotyping or miscarriages

Palomaki 2007 FTS

Clinical features and settings	Routine screening
Participants	10,775 participants Canada - General Hospital October 2003 - November 2004

Palomaki 2007 FTS *(Continued)*

	Pregnant women Mean maternal age 32.3 years (SD 4.6 years) 10-13 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 23 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age FT NT (encouraged to only accept measurements from sonographers with FMF certification) FT PAPP-A (AutoDELFIA, PerkinElmer) FT hyperglycosylated-hCG (Nichols Advantage Specialty system, Nochols Institute Diagnosics)
Follow-up	From electronic record searches of local patient and cytogenetic records and case finding of local and regional birth records
Aim of study	To validate Down's syndrome screening protocols that include hyperglycosylated-hCG measurements
Notes	

Table of Methodological Quality

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

Palomaki 2007 FTS *(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

Perni 2006

Clinical features and settings	Routine screening
Participants	4615 participants USA - single institution January 2003 to September 2004 Pregnant women Singleton pregnancies Mean age 33.0 years (IQR 31.0-36.0) 10-13 weeks' gestation
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 22 cases Reference standards: CVS or amniocentesis. Cytogenetic testing in cases of miscarriage. Follow-up to birth.
Index and comparator tests	Maternal First trimester NT (FMF methods) First trimester PAPP-A and free ßhCG (dried blood spots, methodology described elsewhere)
Follow-up	Outcome information from computerised medical record review. Numbers of patients lost to follow-up not reported
Aim of study	To evaluate the performance of maternal age, fetal NT, PAPP-A and free ßhCG for aneuploidy screening
Notes	Appears that all cases of Down's were diagnosed prenatally by karyotyping

Table of Methodological Quality

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

Perni 2006 *(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	No	No details of withdrawals given

Prefumo 2005

Clinical features and settings	High-risk referral for invasive testing
Participants	544 participants UK - tertiary referral fetal medicine unit December 2001 to November 2003 Pregnant women Median age 37 years (19-46 years) Singleton pregnancies 11-14 weeks' gestation
Study design	Prospective cohort study
Target condition and reference standard(s)	Down's syndrome: 47 cases Reference standard: CVS
Index and comparator tests	Maternal age First trimester NT (methods not reported), risk cut-point 1:300

Prefumo 2005 *(Continued)*

	First trimester nasal bone examination (mid-sagittal view with beam of the ultrasound transducer being parallel to the nasal bones, previously described) First trimester ductus venous flow (abnormal defined as absent or reversed flow. Angle of insonation < 30 degrees. 3 minutes allotted time. NB previously described)
Follow-up	100% karyotyping
Aim of study	To assess the role of fetal ductus venous and nasal bone evaluation in first trimester screening for Down's syndrome
Notes	

Table of Methodological Quality

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	CVS
Partial verification avoided? All tests	Yes	All women received a reference standard
Differential verification avoided? All tests	Yes	All women had the same reference standard
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	Not possible to satisfactorily assess ductus venous flow in 4 cases (0.6%) and nasal bones in 52 cases (8.3%)
Withdrawals explained? All tests	Yes	158 patients not included in the study due to time restrictions or due to the patient declining taking part

Prefumo 2006

Clinical features and settings	Routine screening
Participants	7116 participants UK - single institution December 2001 to November 2003 Pregnant women Singleton pregnancies Mean age 31.4 years (14.5-50.2 years) 10-14 weeks' gestation
Study design	Prospective 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 First trimester NT (all patients) (mid-sagittal view) First trimester nasal bone assessment
Follow-up	Outcome information from computerised hospital records. Results cross-matched with the registry of the Regional Genetics Service. No report of how many patients lost to follow-up
Aim of study	To assess the role of fetal nasal bone evaluation in first trimester screening for trisomy 21 in selected and unselected pregnancies
Notes	

Table of Methodological Quality

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

Prefumo 2006 *(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	Yes	Nasal bones could not be satisfactorily assessed in 9.9% of fetuses
Withdrawals explained? All tests	No	No details of withdrawals given

Ramos-Corpas 2006

Clinical features and settings	Routine screening
Participants	1800 participants Spain - hospital fetal medicine department June 2003 to April 2004 Pregnant women Singleton pregnancies Mean age 30.1 years (15-46 years) (SD 5.37), 18% ≥ 35 years First trimester (before week 14)
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 7 cases Reference standards: invasive testing offered to patients considered high risk at screening (> 1:300) or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF method (Accuson XP10, Mountain View, California) Maximum allotted time of 20 minutes) First trimester nasal bone assessment (in 93.4% of patients) Risk cut-point 1:300 PAPP-A and free ßhCG (Delfia Xpress 6000 immunoanalyzer, Perkin Elmer) - not used in study Published population parameters used (Wald 2003)

Ramos-Corpas 2006 *(Continued)*

Follow-up	Follow-up in all patients without invasive testing by 1) monitoring all births and miscarriages at the hospital, 2) continued contact with the genetics departments and 3) telephone follow-up. States in abstract that only fetuses with complete follow-up results included in the study
Aim of study	To evaluate the utility of determining the presence or absence of nasal bone in a low-risk fetal population
Notes	5 cases diagnosed by invasive testing, 2 by follow-up

Table of Methodological Quality

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	Nasal bones could not be satisfactorily assessed in 6.6% of fetuses
Withdrawals explained? All tests	No	No details of withdrawals given

Rissanen 2007

Clinical features and settings	Routine screening
Participants	4776 participants undergoing NT and/or biochemical screening

Rissanen 2007 *(Continued)*

	Finland - hospitals or health care centres 1999 - 2000 Pregnant women Singleton pregnancies Mean maternal age 29.5 years, 17.7% ≥ 35 years 10-13 weeks' gestation
Study design	Prospective cohort
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 (Trained personnel) First trimester PAPP-A and free ßhCG (AutoDelfia kits, PerkinElmer) Risk cut-point 1:250
Follow-up	Outcomes obtained from all maternity clinics, the Finnish Register of Congenital Malformations and the National Research and Development Centre for Welfare and Health. Follow-up was complete in 99% of live-born infants. Data on miscarriages (n = 68) received from the National Research and Development Centre for Welfare and Health
Aim of study	To evaluate whether first trimester screening markers are altered in pregnancies affected both by other chromosomal defects than trisomy 21 and structural anomalies and whether it is possible to detect these pregnancies by combined ultrasound and biochemical screening
Notes	

Table of Methodological Quality

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?	No	Reference standard interpreted with knowledge of index test results

Rissanen 2007 *(Continued)*

All tests		
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 to 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, or 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 the method of follow-up not given. 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
Notes	Includes cost-effectiveness analysis

Rozenberg 2002 *(Continued)*

Table of Methodological Quality

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 had 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

Rozenberg 2007

Clinical features and settings	Routine screening
Participants	14,934 participants Canada - multicentre study Pregnant women Singleton pregnancies Mean maternal age 30.9 (SD 4.5) years 11-13 weeks' gestation

Rozenberg 2007 *(Continued)*

Study design	Prospective cohort study
Target condition and reference standard(s)	Down's syndrome: 51 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (trained assessors following protocol) First trimester PAPP-A and free ßhCG (PerkinElmer Life Sciences) Second trimester ultrasound and/or serum markers (free ßhCG and AFP or total hCG, AFP and uE3) performed in some cases Risk cut-point 1:250
Follow-up	Notebooks in maternity hospitals used to record information on patient characteristics, screening and outcome at birth. Data obtained from cytogenetic laboratories and DASDY database (contains results of birth examinations). Letters sent to women with missing outcome information and, after 3 months, if there was no response, they were contacted by telephone
Aim of study	To evaluate the performance, acceptability and cost-effectiveness ratio of a pragmatic approach to screening for Down's syndrome based on the combined first trimester test supplemented by routine ultrasound at 20-22 weeks in the general population
Notes	

Table of Methodological Quality

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?	Yes	Information available as would be in standard clinical practice

Rozenberg 2007 *(Continued)*

All tests		
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	Yes	554 women (3.7%) did not undergo screening

Sahota 2010

Clinical features and settings	Routine screening
Participants	10,854 pregnancies with complete outcome data China - University Hospital January 2005 - May 2008 Pregnant women Singleton pregnancies Median maternal age 33.1 years, 30.1% of women aged ≥ 35 years 10-13 weeks' gestation
Study design	Retrospective cohort
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 First trimester NT (FMF accredited sonographers, HDI 5000, Philips Medical System) First trimester PAPP-A and free ßhCG (kryptor analyser, Brahms Diagnostica GmbH) Contingent screening strategies • Strategy-NT-BC: combined screening and if risk intermediate, nasal bone assessment added • Strategy-BC: screening with PAPP-A and free ßhCG and if intermediate risk, NT added. If risk still intermediate, nasal bone assessment added • Strategy-NT: screening with NT and if intermediate risk, PAPP-A and free ßhCG added. If risk still intermediate, nasal bone assessment added Intermediate risk cut-points 1:50 to 1:1000
Follow-up	Fetal karyotypes entered into database when available. Data on pregnancy outcomes obtained either from local maternity database for those who delivered in the unit or via telephone calls to patients
Aim of study	To assess the relative performance of a multi-stage first trimester screening protocol for fetal Down's syndrome
Notes	

Sahota 2010 *(Continued)*

Table of Methodological Quality

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

Salomon 2010

Clinical features and settings	Routine screening
Participants	21,492 participants France - Single Health Authority district January 2001 - December 2003 Pregnant women Median maternal age 30.7 years (18.0-46.3 years) 11-13 weeks' gestation

Salomon 2010 *(Continued)*

Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 80 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (sonographers trained to FMF standards) First trimester PAPP-A and free ßhCG (time resolved fluorescent assay, PerkinElmer Life Sciences) Routine abnormality scan for structural malformations (20-24 weeks) Femur length at routine abnormality scan
Follow-up	Case report forms completed by attending obstetrician or midwife throughout pregnancy and delivery. Databases of certified laboratories cross-checked with delivery and outcome data in all maternity units, the databases of all cytogenetic laboratories, the database of the health authority (DASDY), contact with women by mail 3 months after expected delivery and direct telephone with women.
Aim of study	To evaluate the performance of the contingent use of femur length at routine mid-trimester scan in screening for Down's syndrome in women having previously undergone first trimester screening with disclosure of risk estimates
Notes	

Table of Methodological Quality

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

Salomon 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

Santiago 2007

Clinical features and settings	Routine screening
Participants	4248 participants Spain - Screening database managed by the Fetaltest project To December 2005 Pregnant women Singleton pregnancies Mean maternal age 30.6 years (14-46 years) 11-13 weeks' gestation
Study design	Retrospective cohort
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 (trained sonographers) First trimester PAPP-A and free ßhCG (details not reported) Cut-point 1:300 and detection rate at 5% FPR
Follow-up	Follow-up by supervising the live births and miscarriages at the Hospital together with continuous contact with the genetics department. 24 pregnancies ended in miscarriage and were lost to follow-up. In 269 women not giving birth at that Hospital, only those karyotyped were followed up. In total, 287 women (6.8%) were lost to follow-up
Aim of study	To determine whether delta-NT could be extrapolated successfully from 1 centre-specific NT reference curve to another and thus to empirically calculate the likelihood ratios of delta-NT
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population

Santiago 2007 *(Continued)*

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

Sau 2001

Clinical features and settings	Routine screening
Participants	3185 participants UK - single hospital November 1996 to November 1998 Pregnant women Mean age 28 years (SD 5) 11-14 and 6-20 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down’s syndrome: 8 cases Reference standards: invasive testing (women with high risk on screening) or follow-up to birth

Sau 2001 *(Continued)*

Index and comparator tests	Maternal age First trimester NT (FMF methods, transabdominal route) in 84% of women. NT risk cut-point of 1:100 or if NT measurement > 95th centile for that particular CRL considered screen positive. Confirmatory NT test conducted in all women positive on first NT screening Second trimester AFP, ßhCG and uE3 in 49% of women. Serum risk cut-point 1:250
Follow-up	Follow-up from computerised maternity records, the neonatal database and the hospital termination of pregnancy and miscarriage record books
Aim of study	To present data on the performance of biochemical screening in a population with a prior low-risk screening result
Notes	

Table of Methodological Quality

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	In 122 (4.3%) of women, a second NT scan was needed since the first 1 failed to obtain a measurement
Withdrawals explained? All tests	Yes	Of 3704 women booked for hospital delivery, 3185 had at least 1 screening test and were included in the study

Schaelike 2009

Clinical features and settings	Routine screening
Participants	10,668 participants with complete outcome data Germany - private centre November 2000 - December 2006 Pregnant women Singleton pregnancies Maternal age ≥ 35 years in 31.0% of women 11-13 weeks' gestation
Study design	Prospective cohort
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 (FMF certified physicians) First trimester PAPP-A and free ßhCG (Kryptor analyser, Brahms GmbH) Cut-point 1:300
Follow-up	Information provided by either obstetric departments or obstetricians. Results from CVS and amniocentesis, as well as karyotypes from aborted fetal tissue or from postnatal investigations were used. 3.9% of women were lost to follow-up and were excluded from the study
Aim of study	To assess the performance of a combined first trimester screening concept for trisomies 21, 18 and 13 applied to a low- and high-risk patient sample in a specialised private centre for prenatal medicine
Notes	

Table of Methodological Quality

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?	Yes	Reference standard was independent of the index test

Schaelike 2009 *(Continued)*

All tests		
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

Schielen 2006

Clinical features and settings	Routine screening
Participants	4033 participants The Netherlands - multicentre (44 centres) study July 2002 to May 2004 Singleton pregnancies Pregnant women aged 18-47 years (median 36.5 years) 10-14 weeks' gestation
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 21 cases Reference standards: invasive testing or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF methods) First trimester free ßhCG and PAPP-A (AutoDELFIA analyser)
Follow-up	Women were asked to fill in a questionnaire about outcome of pregnancy. A second request was sent by mail if necessary 784 patients were lost to follow-up (16.2%) and were excluded from the study
Aim of study	To report the results of a first trimester combined-test screening programme in a multicentre routine clinical setting
Notes	

Schielen 2006 *(Continued)*

Table of Methodological Quality

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

Schuchter 2001

Clinical features and settings	Routine screening
Participants	9342 participants Austria - single institution January 1994 to December 1998 Pregnant women Singleton pregnancies Mean age 28 years (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 mm, 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 (5-MHz transducer, Acuson Corp) Second trimester AFP, uE3 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. 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
Notes	

Table of Methodological Quality

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

Schuchter 2001 *(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

Schuchter 2002

Clinical features and settings	Routine screening
Participants	4802 participants Austria - single institution December 1997 to April 2000 Singleton pregnancies Pregnant women 13.0% > 35 years 10-12 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 14 cases Reference standards: CVS and amniocentesis (offered to patients with increased risk (> 1:400) at first trimester screening. CVS recommended when NT > 3.5 or when women did not want to wait until the 15th week for amniocentesis), or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (transabdominal transducer, 5-MHz curvilinear Transducer, Acuson, Mountain View), cut-point 2.5 mm First trimester PAPP-A and free ßhCG (done radioimmunologically, kits by Ortho Clinical Diagnostics) Combined risk cut-point 1:250
Follow-up	Patients without follow-up information (n = 92, 2%) were excluded from the study. 27 women with spontaneous abortions were also excluded from the study
Aim of study	To determine the detection rate of the combined test, NT alone and maternal age alone in a non-selected population at a false positive rate of about 5%
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population

Schuchter 2002 *(Continued)*

Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth
Partial verification avoided? All tests	Yes	All patients 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	Women not attending visits were excluded from the study

Schwarzler 1999

Clinical features and settings	Routine screening
Participants	4523 participants UK - single institution July 1996 to November 1997 Pregnant women Mean age 29.4 years (16-47 years) 10-14 weeks' gestation
Study design	Prospective consecutive cohort
Target condition and reference standard(s)	Down's syndrome: 12 cases Reference standards: invasive testing (women considered high risk on screening) or follow-up to birth

Schwarzler 1999 *(Continued)*

Index and comparator tests	Maternal age NT (Sagittal plane by transabdominal (92.7%) and transvaginal (7.3%) sonography) Adjusted risk cut-point 1:270
Follow-up	Pregnancy outcome obtained via questionnaires, examination by neonatologist and outcome cross-referenced with regional cytogenetics registry. 26 test-negative patients lost to follow-up and excluded from the study
Aim of study	To evaluate first trimester pregnancy screening for fetal aneuploidy and congenital heart defects by maternal age and NT measurement in an unselected population
Notes	3 live births, 9 termination of pregnancy

Table of Methodological Quality

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

Scott 2004

Clinical features and settings	Routine screening
Participants	2053 participants Australia - private practice (Sydney Ultrasound for Women) July 2000 to May 2002 Pregnant women Median age 32 years (15-44 years), 29% ≥ 35 years Singleton pregnancies 11-14 weeks' gestation
Study design	Prospective cohort study
Target condition and reference standard(s)	Down's syndrome: 5 affected cases Reference standards: invasive testing or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF methods, sagittal plane, ATL 5000; Philips) First trimester free ßhCG and PAPP-A (kryptor analyser, Brahms Diagnostics) All participants had all tests Risk cut-point 1:300
Follow-up	Data obtained from referring doctors or patients via letter, phone or completed feedback form given at the time of consultation. Only cases of known outcome included in the study. 68 (1.3%) lost to follow-up, largely due to miscarriage (n = 20) and loss to follow-up (n = 40).
Aim of study	To report the sensitivity of combined first trimester biochemistry and ultrasound screening for Down's syndrome in an Australian private practice specialising in obstetric ultrasound
Notes	Only women having biochemical testing before NT were included in the study. This was done to avoid bias from women declining biochemical testing following negative NT.

Table of Methodological Quality

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?	No	Choice of reference standard depended on index test results

Scott 2004 *(Continued)*

All tests		
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

Sepulveda 2007

Clinical features and settings	Routine screening
Participants	1287 participants Chile - fetal medicine centre January 2003 - January 2006 Pregnant women Median maternal age 33 years (range 14-47 years), 35.4% ≥ 35 years Singleton pregnancies 11-14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 31 cases Reference standards: CVS, amniocentesis, cordocentesis or follow-up to birth
Index and comparator tests	Maternal age First trimester NT and nasal bone assessment (Accuvix XQ, Medison or Voluson 730, GE Healthcare) (only included in study if scanned by 1 of 2 fetal medicine specialists following FMF guidelines)
Follow-up	Cases of chromosomal abnormality were identified from the cytogenetics laboratory logbook, which recorded all the cytogenetic studies performed prenatally, after a spontaneous abortion or fetal death, or in neonates with physical abnormalities. Information from the remaining cases was obtained from the delivery records and neonatal discharge summaries, which recorded the condition of the neonate at birth and the physical examination performed by a neonatologist

Sepulveda 2007 *(Continued)*

Aim of study	To report their experience with first trimester screening for trisomy 21 by using the combination of NT thickness and nasal bone assessment
Notes	

Table of Methodological Quality

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

Snijders 1998

Clinical features and settings	Routine screening
Participants	96,127 participants UK - multicentre study (22 centres) Women due to deliver before June 1997

Snijders 1998 *(Continued)*

	Pregnant women Median age 31 years (14-49 years) Singleton pregnancies 10 to 14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 326 cases Reference standards: CVS and amniocentesis (9.6% of women) or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (sagittal section) Risk cut-point 1:300
Follow-up	Each women given a request form to complete about the outcome of pregnancy. 4184 women (4.2%) were excluded due to loss to follow-up or due to miscarriages that were not karyotyped
Aim of study	To investigate the assessment of risk by a combination of maternal age and fetal NT thickness, measured by ultrasonography at 10-14 weeks of gestation
Notes	

Table of Methodological Quality

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?	Yes	Information available as would be in standard clinical practice

Snijders 1998 *(Continued)*

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

Sorensen 2011

Clinical features and settings	Routine screening
Participants	19,694 participants Denmark - 2 centres July 2005 - June 2007 Pregnant women Singleton pregnancies Maternal age: healthy mean age 30.4 years (16-45 years), 16.5% ≥ 35 years, Down's syndrome median age 34 years (23-44 years) 8-13 weeks' gestation
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 100 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF certified sonographers) First trimester PAPP-A and free ßhCG (TRACE technology, Kryptor instrument, Brahms AG)
Follow-up	Details not reported. It was stated that, for non-Down's syndrome pregnancies, only those with known healthy fetus were included
Aim of study	To develop 2 alternative risk calculation programmes to assess whether the screening efficacies for T13, T18 and T21 could be improved by using our locally estimated medians
Notes	

Table of Methodological Quality

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

Sorensen 2011 *(Continued)*

All tests		
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

Spencer 1999

Clinical features and settings	Women referred for invasive testing or self-referred for screening
Participants	1156 participants: 210 cases and 946 controls matched for gestational and maternal age UK - fetal medicine research centre Dates not specified Pregnant women Median maternal age 38 years (19-46 years) (cases) and 36 years (15-47 years) (controls) 10-14 weeks' gestation
Study design	Case-control study
Target condition and reference standard(s)	Down's syndrome: 210 cases Reference standards: invasive testing (high-risk women) or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (methods not reported)

Spencer 1999 *(Continued)*

	Frozen serum samples tested for: First trimester free ßhCG and PAPP-A (Kryptor analyser, time resolved amplified cryptate emission (TRACE))
Follow-up	Stated that pregnancy outcome was ascertained in all women
Aim of study	To examine the potential impact of combining maternal age with fetal NT thickness and maternal serum free ßhCG and PAPP-A in screening for trisomy 21 at 10-14 weeks of gestation
Notes	

Table of Methodological Quality

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	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	Unclear	Unclear if all index tests 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

Spencer 2002

Clinical features and settings	Routine screening

Spencer 2002 *(Continued)*

Participants	278 participants: 54 cases and 224 controls (no details of how selected) UK - OSCAR screening program Samples collected since 1998 Pregnant women Median maternal age 36 years (20-44 years) (cases) and 30 years (16-41 years) (controls) 11-13 weeks' gestation
Study design	Case-control study
Target condition and reference standard(s)	Down's syndrome: 54 cases Reference standards: details not reported
Index and comparator tests	Maternal age First trimester NT (FMF methods) Frozen serum samples tested for: First trimester free ßhCG, PAPP-A and ThCG (Kryptor Analyser (TRACE) and automated immunofluorescent assays)
Follow-up	Methods for follow-up to birth not reported
Aim of study	To assess serum hyperglycosylated hCG for use in the first trimester of pregnancy as a marker of Down's syndrome
Notes	

Table of Methodological Quality

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 (Nicolaides 2005(OSCAR screening program))
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

Spencer 2002 *(Continued)*

Index test results blinded? All tests	Unclear	Unclear of all index tests 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

Spencer 2008

Clinical features and settings	Routine screening
Participants	622 participants: 55 cases and 567 controls matched for gestational age Denmark - screening programme Dates not reported Pregnant women Median maternal age cases 35.8 years, controls 29.3 years 8-13 weeks' gestation (results modelled on only cases where testing conducted before 10 weeks' gestation)
Study design	Case-control study
Target condition and reference standard(s)	Down's syndrome: 55 cases (31 tested before 10 weeks' gestation) Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (details not reported) Fresh serum samples tested for: First trimester PAPP-A and free ßhCG (Kryptor analyser, Brahms) Frozen samples tested for: First trimester ADAM 12 (measured blind to clinical outcome) (manual DELFIA assay, PerkinElmer Life & Analytical Sciences)
Follow-up	Details not reported
Aim of study	To establish the effectiveness or otherwise of ADAM 12 as an early screening marker
Notes	

Table of Methodological Quality

Spencer 2008 *(Continued)*

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

Stenhouse 2004

Clinical features and settings	Routine screening
Participants	5000 participants UK - maternity clinic Over a 3 year period - dates not specified Pregnant women Singleton pregnancies Median age 32 years (14-45 years), 27% ≥ 35 years 11 to 14 weeks' gestation

Stenhouse 2004 *(Continued)*

Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 15 cases Reference standards: invasive testing offered to women with screening risk of > 1:250 or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF methods, ATL HDI 3500, ATL HDI 3000, Toshiba SSA-340A and Kretz Voluson) First trimester free ßhCG and PAPP-A (Clotted venous blood samples, AutoDELFIA immunoassy, Perkin Elmer)
Follow-up	Details not reported
Aim of study	To assess the effectiveness of combined ultrasound and biochemical screening for chromosomal abnormalities in singleton pregnancies in a routine antenatal clinic and laboratory setting
Notes	Fetal loss rate for invasive testing was 1.4% (3/212)

Table of Methodological Quality

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	Yes	NT not successfully measured in 25 patients (0.5%)
Withdrawals explained?	No	No details of withdrawals given

Stenhouse 2004 *(Continued)*

All tests	

Strah 2008

Clinical features and settings	Routine screening
Participants	7096 participants with information available on pregnancy outcome Slovenia - 2 outpatient clinics November 1999 - May 2006 Pregnant women Singleton pregnancies Median maternal age 28.6 years (range 15-42 years), 2.5% ≥ 36 years 11-14 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 First trimester NT (2 FMF certified sonographers) (3.5-5 MHz and 8-4 MHz transducers Toshiba Corevision Pro and 2-5 MHz and 9.3-3.7 MHz transducers GE Healthcare Voluson 730 Pro) Cut-off 1/300
Follow-up	Pregnancy outcomes were obtained from participating women, referring gynaecologists, paediatricians and maternity units and were missing in 3% (n = 225) of cases. Karyotype results were reported from the cytogenetics laboratory. Only women with known outcome were included in the study analysis
Aim of study	To evaluate screening for trisomy 21 by maternal age and NT in a low-risk population
Notes	

Table of Methodological Quality

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

Strah 2008 *(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

Theodoropoulos 1998

Clinical features and settings	Routine screening
Participants	4611 women due to deliver before July 1996 Greece - 4 medical centres Dates not specified Singleton pregnancies Median maternal age 29 years (16-48 years), 7.8% ≥ 37 years 10 to 14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 10 cases Reference standards: CVS or amniocentesis or follow-up to birth. Unclear reference standard in cases of intrauterine death, miscarriages and terminations
Index and comparator tests	Maternal age First trimester NT (FMF methods, transabdominally with 5 or 3.5 MHz curvilinear translucer or transvaginally with 5 MHz transducer) Pandya's risk criteria

Theodoropoulos 1998 *(Continued)*

Follow-up	Results of fetal karyotyping and pregnancy outcome were entered into the database when they became available
Aim of study	To evaluate first trimester screening for chromosomal defects by fetal NT thickness at 10-14 weeks of gestation in 4 medical centres in Greece
Notes	1 set of parents continued with diagnosed Down's pregnancy to birth, 9 terminated. 1 case of Down's syndrome only detected at birth.

Table of Methodological Quality

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

Thilaganathan 1999

Clinical features and settings	Routine screening
Participants	9802 participants

Thilaganathan 1999 *(Continued)*

	UK - district general hospital November 1994 to November 1998 Pregnant women Singleton pregnancies Mean age 29 years (15-45 years) 10 to 14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 21 cases Reference standards: CVS (offered to patients considered high risk on screening) or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (transabdominally, Toshiba SSA-250, Accuson 128XP/4 or Aloka 650CL with 3.5-7.5 curvilinear transducers)
Follow-up	Pregnancy outcomes from hospital records and general practitioners. Karyotype results or postnatal tests were provided by the local Regional Cytoenetics laboratory. The proportion of patients who were followed up is not reported (49 patients had not given birth at the time of analysis of outcomes)
Aim of study	To evaluate the effectiveness of 10-14 week NT measurement in routine ultrasounds screening for Down's syndrome
Notes	

Table of Methodological Quality

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

Thilaganathan 1999 *(Continued)*

Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	Yes	Unsuccessful NT in 10.1% of patients
Withdrawals explained? All tests	Yes	Patients not included due to ineligibility described

Timmerman 2010

Clinical features and settings	High-risk referral
Participants	445 fetuses with increased risk based on NT or biochemical testing and information available on pregnancy outcome The Netherlands - fetal medicine unit September 1996 - March 2008 Mean maternal age 34.5 years (19-45 years) First trimester
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 72 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	First trimester ductus venosus pulsatility index and Ductus venosus a-wave (methods reported elsewhere)
Follow-up	Pregnancy outcome was obtained from standard follow-up forms filled in and returned by patients, maternity wards or midwife practices and by reviewing neonatal, pathology and clinical paediatric notes. When the baby was born without structural defects or dysmorphic features, the chromosomes were assumed to be normal. In all cases of enlarged NT or antenatal suspicion of abnormal development, the infant was investigated by a neonatologist, paediatric cardiologist or geneticist.
Aim of study	To investigate if ductus venosus pulsatility index for veins and a-wave measurements can increase the accuracy of first trimester Down's syndrome screening in a high-risk population
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice
Acceptable reference standard?	Yes	Karyotyping or follow-up to birth

Timmerman 2010 *(Continued)*

All tests		
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	Satisfactory waveform measurements made in 98% of cases
Withdrawals explained? All tests	No	No details of withdrawals given

Torring 2010

Clinical features and settings	Routine screening
Participants	691 participants: 46 cases and 645 controls Denmark - nationwide screening programme Dates not reported Pregnant women Singleton pregnancies Mean maternal age cases 35 years, controls 31 years 8-11 weeks' gestation
Study design	Case-control study
Target condition and reference standard(s)	Down's syndrome: 46 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age

Torring 2010 *(Continued)*

	First trimester NT (11-13 weeks' gestation) (FMF certified sonographers) First trimester PAPP-A and free ßhCG (fresh serum, 8-11 weeks' gestation) (Kryptor analyser, Brahms) First trimester ADAM 12 (frozen serum, 8-11 weeks' gestation) (Kyptor analyser, assay by Cezanne SAS, TRACE technology)
Follow-up	Not reported
Aim of study	To determine whether ADAM 12 is a useful serum marker for fetal trisomy 21 using the mixture model
Notes	

Table of Methodological Quality

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 some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests 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

Vadiveloo 2009

Clinical features and settings	Routine screening
Participants	10,189 participants UK - screening programme July 2000 - October 2005 Pregnant women Median maternal age 33.1 years, 36.9% ≥ 35 years 9-14 weeks' gestation
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 44 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (trained sonographers) First trimester PAPP-A and free ßhCG (DELFIA fluoroimmunoassay, PerkinElmer LAS) Contingent: biochemistry high risk cut-off 1:42, low risk cut-off 1:1000. If biochemical/maternal age risk between 1:42 and 1:1000, NT results added and combined risk calculated. Final cut-point 1:250
Follow-up	Not reported
Aim of study	To assess the performance of a 2-stage screening protocol for Down's syndrome based on initial serum marker analysis for all women and NT measurement only in women with intermediate risks
Notes	

Table of Methodological Quality

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

Vadiveloo 2009 *(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

Valinen 2007

Clinical features and settings	Routine screening
Participants	7534 participants Finland - screening programme 2002-2004 Pregnant women Singleton pregnancies Mean maternal age 29.6 years, 18.6% ≥ 35 years 10-12 weeks' gestation
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 30 cases (24 underwent NT as well as biochemical testing) Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (trained nurses, midwives and doctors) (4765 women) First trimester PAPP-A and free ßhCG (details not reported) (all women) Cut-point 1:250
Follow-up	Contacted chromosome laboratory at the department of clinical genetics in the Oulu university clinic and the Finish Register of Congenital Malformation and the National Research and Development Centre for Welfare and Health
Aim of study	To compare the efficacy of both separate and combined maternal serum testing and fetal NT measurement in the first trimester screening for Down's syndrome in northern Finland

Valinen 2007 *(Continued)*

Notes

Table of Methodological Quality

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

Viora 2003

Clinical features and settings	Routine screening
Participants	1752 participants Italy - ultrasound and prenatal diagnosis unit December 2001 to June 2002 Pregnant women Median age 32 years (18-47 years)

Viora 2003 *(Continued)*

	11 to 14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 10 cases Reference standards: CVS or follow-up to birth
Index and comparator tests	Maternal age Nasal bone assessment (ultrasound examinations with Aloka SSD-1700 or ATL-Philips 5000 HCD)
Follow-up	Follow-up to birth in all cases of abnormalities. Not reported if there was follow-up in screen-negative patients
Aim of study	To evaluate the significance of nasal bone ossification as a marker fir trisomy 21 at 11 to 14 weeks' gestation in an unselected population
Notes	

Table of Methodological Quality

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	In 154 cases (8.1%) fetal profile was not obtained
Withdrawals explained?	No	No details of withdrawals given

Viora 2003 *(Continued)*

All tests	

Wald 2003

Clinical features and settings	Routine screening
Participants	39,983 participants UK and Austria - multicentre 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: 85 cases Reference standards: invasive testing (following second trimester screening) or follow-up to birth
Index and comparator tests	First trimester NT (midsaggital section, optimal magnification of thickness of translucent space between inner skin surface and fascia covering cervical spine (white black interface (oute) - black white interface (inner), 41 models of ultrasound machine, 20 minutes allotted scanning time) First and second trimester serum AFP, hCG, uE3, PAPP-A, free ßhCG (time resolved fluoroimmunoassay, AutoDELFIA) First and second trimester inhibin A (Sandwich enzyme linked immunosorbent assay, Oxford bioinnovation) First 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. 4% of total patient cohort did not have a documented outcome of pregnancy. Unclear if any of these were included in the nested case-control study
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
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

Item	Authors' judgement	Description
Representative spectrum?	Yes	Routine screening of typical pregnant population

Wald 2003 *(Continued)*

All tests		
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	Unclear	Unclear if all index tests 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

Wapner 2003

Clinical features and settings	Routine screening
Participants	8216 participants USA multicentre study (12 prenatal diagnostic centres) Dates not specified Singleton pregnancies Pregnant women Mean age 35 years (SD 4.6), 50% ≥ 35 years 11 to 14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 61 cases

Wapner 2003 *(Continued)*

	Reference standards: invasive testing. Miscarriage with cytogenetic testing. Follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF methods) Dried blood samples tested for: First trimester free ßhCG and PAPP-A (dried blood samples, enzyme-linked immunoadsorbent assay as previously described) Risk cut-point 1:270
Follow-up	Follow-up to birth by directly following up women and reviewing delivery records. An effort was also made to obtain information on terminated or miscarried pregnancies. 196 (2.3%) of patients without follow-up information were excluded and women with a previous trisomy 18 or 21 pregnancy were also excluded
Aim of study	To evaluate the use of combined first trimester markers for aneuploidy in clinical practice
Notes	16 live Down's syndrome births

Table of Methodological Quality

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

Wapner 2003 *(Continued)*

Withdrawals explained? All tests	No	No details of withdrawals given

Wax 2009

Clinical features and settings	Routine screening
Participants	2231 participants USA January 2005 - January 2008 Pregnant women Singleton pregnancies Mean maternal age 36.7 years (SD 3.2 years) First and second trimester
Study design	Retrospective cohort
Target condition and reference standard(s)	Down's syndrome: 8 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (Sonographers credited by FMF or Nuchal Translucency Quality Review Program) First trimester PAPP-A and free ßhCG (details not reported) Second trimester ultrasound (in 884 women) Cut-point for combined test 1:220
Follow-up	Down's syndrome cases ascertained from pre-natal genetic database, including prenatal and newborn testing or physical examination at birth
Aim of study	To evaluate the trisomy 21 screening performance of the first trimester combined test followed by second trimester genetic sonography
Notes	

Table of Methodological Quality

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

Wax 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	No	No details of withdrawals given

Wojdemann 2005

Clinical features and settings	Referrals for screening
Participants	8622 participants Denmark - 3 obstetrics departments March 1998 to June 2001 Pregnant women Mean age 29 years, 10.8% ≥ 35 years Singleton pregnancies 11 to 14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 12 cases Reference standards: invasive testing (in cases of increased risk) or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF methods, Logic 700 MR machine) (all women)

Wojdemann 2005 *(Continued)*

	First trimester free ßhCCG (AFP/ßhCG Auto Delfia kit) and PAPP-A (In-house ELISA (Sandwich)) in 6,441 women (75%) Risk cut-point 1:250
Follow-up	Cross-checking with all the chromosome laboratories in Denmark. Follow-up in 96.2% of pregnancies through patients records
Aim of study	To determine the performance of screening for Down's syndrome and other major chromosomal abnormalities using NT, free ßhCG and PAPP-A in a prospective study of a non-selected population
Notes	Uptake of screening was 73% (9,941 accepted out of 13,621 offered screening) Women with miscarriages excluded from the study 3 live Down's syndrome births

Table of Methodological Quality

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	Yes	NT could not be measured in 2.5% of cases
Withdrawals explained? All tests	No	No details of withdrawals given

Wortelboer 2009

Clinical features and settings	Routine screening
Participants	20,293 participants with complete outcome data The Netherlands - nationwide screening programme May 2004 - July 2006 Pregnant women Singleton pregnancies Median maternal age 34.9 years (15-48 years) 8-14 weeks' gestation
Study design	Cohort
Target condition and reference standard(s)	Down's syndrome: 87 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF protocols) First trimester PAPP-A and free ßhCG (AutoDELFIA analyser, PerkinElmer, Turku) Cut-point for combined test 1:250
Follow-up	Pregnancy outcome was evaluated by questionnaire and collected through self-reporting of the participating women. Due to strict privacy rules of the Dutch Personal Data Protection Act, the researchers were allowed to send a reminder letter to collect missing data only once. Women without complete information on outcome were excluded from the study
Aim of study	To study the performance of the first-trimester combined test between 2004 and 2006 compared to a previous period to investigate changes in time and identify reasons for sub-optimal performance
Notes	

Table of Methodological Quality

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

Wortelboer 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	Yes	Only 65% of biochemistry screened women (n = 41,782) had NT results

Wright 2008

Clinical features and settings	Routine screening
Participants	37,488 participants with complete outcome data UK - single centre July 1999 - July 2005 Pregnant women Singleton pregnancies Median maternal age 35.2 years (16-52 years) 11-13 weeks' gestation
Study design	Cohort
Target condition and reference standard(s)	Down's syndrome: 264 cases Reference standards: karyotyping or follow-up to birth
Index and comparator tests	Maternal age First trimester NT First trimester PAPP-A and free ßhCG (Kryptor system, Brahms AG)
Follow-up	Maternal characteristics and test results were recorded in a computer database and karyotype results and details on pregnancy outcomes added as they became available. Women without complete outcome data (n = 1231, 3.2%) were excluded from the study
Aim of study	To examine the validity of methods used to derive patient-specific risks form NT measurements

Wright 2008 *(Continued)*

Notes

Table of Methodological Quality

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

Clinical features and settings	Routine screening
Participants	223,361 pregnant women UK, Denmark and Cyprus – multicentre Some data from UK and Denmark in previous publications Dates not reported Singleton pregnancies

Wright 2010 *(Continued)*

	Median maternal age 31.9 years (IQR 27.7-35.8 years) 7-14 weeks' gestation
Study design	Cohort
Target condition and reference standard(s)	Down's syndrome: 886 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 and free βhCG (Kryptor system, Brahms AG or Delfia Express sustem, PerkinElmer, Waltham)
Follow-up	Karyotype results and details on pregnancy outcomes were added to databases as soon as they became available
Aim of study	To establish an algorithm for first trimester combined screening for trisomy 21 with biochemical testing from 7 to 14 weeks' gestation and ultrasound testing at 11-13 weeks
Notes	Taken results modelled for PAPP-A and free ßhcg at 12 weeks as that was most common time for testing (44% of women)

Table of Methodological Quality

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

Wright 2010 *(Continued)*

Uninterpretable results reported? All tests	No
Withdrawals explained? All tests	No

Zoppi 2001

Clinical features and settings	Routine screening
Participants	10,001 participants Italy - genetic diagnosis centre May 1996 to unspecified date Pregnant women Median age 33 years (14-48 years) Singleton pregnancies 10 to 14 weeks' gestation
Study design	Prospective cohort
Target condition and reference standard(s)	Down's syndrome: 64 cases Reference standards: amniocentesis, CVS or follow-up to birth
Index and comparator tests	Maternal age First trimester NT (FMF methods) Risk cut-points of 1:100, 1:200 and 1:300
Follow-up	Outcome obtained from women themselves. 1422 patients (11%) with no data on follow-up outcome and 202 patients with miscarriages were excluded from the study
Aim of study	To examine the distribution of fetal NT thickness in normal and abnormal fetuses in Sardinia and to determine its effectiveness as a screening tool
Notes	Study design unclear (maybe a case-control study)

Table of Methodological Quality

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

Zoppi 2001 *(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	Yes	NT could not be measured in 25 (0.2%) of cases
Withdrawals explained? All tests	No	No details of withdrawals given

AFP:alpha-fetoprotein
ßhCG: beta human chorionic gonadotrophin
CVS: chorionic villus sampling
DELFIA: dual labelled time resolved fluorescent assay
DVPI: ductus venosus pulsivity index
FMF: frontomaxillary facial
hCG: human chorionic gonadotrophin
NT: nuchal translucency
PAPP-A: pregnancy-associated plasma protein A
PIGF: placental growth factor
uE3: unconjugated oestriol

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

Study	Reason for exclusion
Abbas 1995	Unable to extract useful data
Abdul-Hamid 2004	No Down's syndrome pregnancies
Abraha 1999	Unable to extract useful data
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

Study	Reason for exclusion
Agaard-Tillery 2010	Results presented in another study
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 1999b	USS markers greater than 14 weeks' gestation
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

Study	Reason for exclusion
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
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

Study	Reason for exclusion
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 2009	No diagnostic data
Bornstein 2009a	No diagnostic data
Bornstein 2010	No diagnostic data
Borowski 2007	No diagnostic data
Borrell 2007	No follow-up data
Borrell 2009a	Based on SURUSS data - second trimester serum parameters not actually measured
Borruto 2002	Unable to extract useful data
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 1995b	Unable to extract useful data
Canini 2002	No Down's syndrome pregnancies in study population

Study	Reason for exclusion
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
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 2004	No Down's syndrome pregnancies in study population
Cheng 2004a	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 2007a	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 2002	USS at greater than 14 weeks

Study	Reason for exclusion
Comas 2002a	USS at greater than 14 weeks
Comstock 2006	Unable to extract useful data
Conde-Agudelo 1998	Review article
Cowans 2011	No diagnostic data
Crossley 1991	Less than 80% of pregnancies had gestational age confirmation by ultrasound
Crossley 1993	Less than 80% of pregnancies had gestational age confirmation by ultrasound
Crossley 1996	No Down's syndrome pregnancies in study population
Crossley 2002a	Adjustment factors for smokers
Cuckle 1984	Gestational age not confirmed by USS
Cuckle 1987	Gestational age not confirmed by USS
Cuckle 1987a	No gestational age limits given
Cuckle 1990	Paper presenting adjustment factors
Cuckle 1996	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
Del Carmen Saucedo 2009	No follow-up information
DeVore 2001	Second trimester ultrasound
Dhaifalah 2007	Unable to obtain translation

Study	Reason for exclusion
Dhaifalah 2007a	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 1996	Second trimester ultrasound
Drugan 1996a	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 fetuses
Geipel 2010	ST ultrasound
Gekas 2009	Diagnostic data from other studies
Gekas 2011	Diagnostic data from other studies
Gekas 2011a	Diagnostic parameters from other studies
Gerovassili 2007	No diagnostic data

Study	Reason for exclusion
Ghidini 1998	Comparison of male versus female fetuses
Goetzinger 2010	Second trimester ultrasound
Goldie 1995	Fewer than 80% of study population had 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 2004	Less than 80% follow-up
Gyselaers 2004a	Less than 80% follow-up
Gyselaers 2006	Unaffected pregnancies only
Gyselaers 2006a	Unable to extract useful data
Hackshaw 1995	No Down's syndrome pregnancies in population
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

Study	Reason for exclusion
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
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 2007	Not possible to obtain detection rate
Huang 2007a	No diagnostic data

Study	Reason for exclusion
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
Jakobsen 2011	Not Down’s syndrome specific
Jean-Pierre 2005	Review article
Johnson 1991	Gestational 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

Study	Reason for exclusion
Keith 1992	Summary article
Kelekci 2004	Less than 5 Down's syndrome pregnancies in population
Kellner 1995	Less than 5 Down's syndrome pregnancies in population
Kellner 1995a	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
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 2006	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 1999	No Down's syndrome pregnancies in population

Study	Reason for exclusion
Lam 1999a	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
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

Study	Reason for exclusion
Mandryka-Stankewycz 2009	No diagnostic data
Mangione 2001	Abnormal screening results only
Markov 2008	Unable to obtain paper
Maymon 2001	No Down's syndrome pregnancies in study population
Maymon 2001a	No normal test results included therefore unable to extract meaningful data
Maymon 2002	No Down's syndrome pregnancies in study population
Maymon 2004a	No Down's syndrome pregnancies in study population
Maymon 2005a	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	Part of Merz 2011 cohort
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
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

Study	Reason for exclusion
Muhcu 2008	No diagnostic data
Muller 1994	No Down's syndrome pregnancies in study population
Muller 1996a	Unable to extract useful data
Muller 1999	Unable to extract useful data
Muller 2002	Gestational age greater than 24 weeks
Muller 2002a	Unable to extract meaningful data - unable to separate double and triple test data
Muller 2003b	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 1996	No Down's syndrome pregnancies in population
Neveux 1996a	Unable to extract useful data
Ng 2004	Unable to extract useful data
Nicolaides 1992a	Study of outcomes of abnormal NT results
Nicolaides 2000	Review article
Nicolaides 2004	Review article
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 2001b	No Down's pregnancies in study population
Niemimaa 2002	No Down's syndrome pregnancies in population

Study	Reason for exclusion
Niemimaa 2003	No Down's syndrome pregnancies in population
Noble 1997b	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
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

Study	Reason for exclusion
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
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 predicition
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
Raty 2000	No Down's syndrome pregnancies in population
Rembouskos 2004	Unable to extract useful data
Ren 1992	Review article

Study	Reason for exclusion
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
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 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 2010a	Included in Sahota 2010
Salazar 2007	Unable to obtain paper

Study	Reason for exclusion
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
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

Study	Reason for exclusion
Smith 1999	Unable to extract useful data
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 1995a	No Down's pregnancies in population
Spencer 1996b	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
Spencer 1999a	Review
Spencer 1999b	Statistical methods paper
Spencer 2000a	Examination of median shifts rather than an evaluation of screening

Study	Reason for exclusion
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
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 2 different assays - not actual screening evaluation
Spencer 2008a	Unable to extract appropriate data for unaffected pregnancies
Spong 1999	Comparison of male and female fetuses
Staboulidou 2009	No diagnostic data

Study	Reason for exclusion
Stevens 1998	Literature review
Stoll 1992	Review article
Stressig 2011	ST ultrasound
Su 2002a	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	Gestational age not confirmed by USS
Tanski 1999	Information on screen-positive pregnancies only
Thilaganathan 1998	No Down's syndrome pregnancies in study population
Thilaganathan 1999b	Editorial
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

Study	Reason for exclusion
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
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 2003b	No cases
Wald 2003c	Less than 80% had gestational age confirmed by USS
Wald 2006	Modelled on SURRUS data
Wallace 1994	Unable to extract useful data

Study	Reason for exclusion
Wallace 1997	No Down's syndrome pregnancies in study population
Wang 2010	ST ultrasound
Ward 2005	Review article
Watt 1996a	No Down's syndrome pregnancies in study population
Watt 1996b	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
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

Study	Reason for exclusion
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
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 2003a	Inappropriate study design

CVS: chorionic villus sampling
DR: detection rate
FPR: false positive rate
LMP: last menstrual period
NT: nuchal translucency
USS: ultrasound scan

DATA

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

Table Tests. Data tables by test

Test	No. of studies	No. of participants
1 Aberrant right subclavian artery	1	425
2 Frontomaxillary facial angle >95 percentile	1	242
3 Presence of mitral gap	1	217
4 Maxillary bone length, 5% percentile	1	927
5 Tricuspid regurgitation	1	312
6 Iliac angle 90 degrees	1	2032
7 Ductus venosus a-wave reversed	1	378
8 Ductus venosus pulsivity index > 95 percentile	1	378

Test	No. of studies	No. of participants
9 Nasal bone, mixed cut-points	11	48279
10 NT, 2.5 mm	4	11835
11 NT, 3 mm	6	10381
12 NT, 5FPR	3	63885
13 NT, mixed cut-points	13	90978
14 NT and age, risk 1:100	1	10668
15 NT and age, risk 1:250	10	79412
16 NT and age, risk 1:300	23	252811
17 NT and age, 1FPR	4	98453
18 NT and age, 3FPR	4	98453
19 NT and age, 5FPR	22	288853
20 NT and age, mixed cut-points	50	530874
21 NT and nasal bone, Absent NB + NT ≥ 95th centile	1	486
22 Ductus and age, risk 1:250	1	3731
23 Ductus and age, 5FPR	2	3965
24 Ductus and age, mixed cut-points	5	5331
25 Ductus, NT and age, risk 1:100	1	19736
26 Ductus, NT and age, risk 1:250	1	3727
27 Ductus, NT and age, 5FPR	2	3961
28 Ductus, NT and age, mixed cut-points	3	23697
29 Age and nasal bone, mixed cut-points	4	25303
30 Age, NT and tricuspid blood flow, risk 1:100	1	19736
31 Age, NT and nasal bone, risk 1:100	1	19736
32 Age, NT and nasal bone, risk 1:300	4	9963
33 Age, NT and nasal bone, mixed cut-points	5	29699
34 Age, NT, nasal bone and ductus, risk NT>1:300 AND abnormal DV flow AND absent NB	1	544
35 Age, NT, nasal bone, free ßhCG and PAPP-A, 1st trimester, 5FPR	1	20305

Test	No. of studies	No. of participants
36 Age, NT, nasal bone, free ßhCG and PAPP-A, 1st trimester, mixed cut-points	3	41842
37 Age, NT and free ßhCG, 1st trimester, 5FPR	4	4986
38 Age, NT and free ßhCG, 1st trimester, risk 1:240	1	5809
39 Age, NT and free ßhCG, 1st trimester, mixed cut-points	5	10795
40 Age, NT and PAPP-A, 1st trimester, risk 1:100	1	1507
41 Age, NT and PAPP-A, 1st trimester, risk 1:185	1	5809
42 Age, NT and PAPP-A, 1st trimester, 5FPR	3	2498
43 Age, NT and PAPP-A, 1st trimester, mixed cut-points	5	9814
44 Age, NT and total hCG, 1st trimester, 5FPR	1	1110
45 Age, NT and AFP, 1st trimester, 5FPR	1	1110
46 Age, NT and ITA, 1st trimester, 5FPR	1	278
47 Age, NT and inhibin, 1st trimester, risk 1:100	1	40
48 Age, NT and inhibin, 1st trimester, risk 1:250	1	40
49 Age, NT and inhibin, 1st trimester, risk 1:400	1	40
50 Age, NT and inhibin, 1st trimester, 5FPR	1	1110
51 Age, NT and inhibin, 1st trimester, mixed cut-points	2	1150
52 Age, NT, PAPP-A and free ßhCG, 1st trimester, risk 1:100	10	102332
53 Age, NT, PAPP-A and free ßhCG, 1st trimester, risk 1:150	5	177643
54 Age, NT, PAPP-A and free ßhCG, 1st trimester, risk 1:200	8	135768
55 Age, NT, PAPP-A and free ßhCG, 1st trimester, risk 1:220	1	2231
56 Age, NT, PAPP-A and free ßhCG, 1st trimester, risk 1:250	25	174712
57 Age, NT, PAPP-A and free ßhCG, 1st trimester, risk 1:300	29	544681
58 Age, NT, PAPP-A and free ßhCG, 1st trimester, 1FPR	7	88874
59 Age, NT, PAPP-A and free ßhCG, 1st trimester, 3FPR	9	312680
60 Age, NT, PAPP-A and free ßhCG, 1st trimester, 5FPR	24	391874
61 Age, NT, PAPP-A and free ßhCG, 1st trimester, mixed cut-points	69	1173853
62 Age, NT, PAPP-A and uE3, 1st trimester, 5FPR	1	576
63 Age, NT, PAPP-A and ITA, 1st trimester, 5FPR	2	11053

Test	No. of studies	No. of participants
64 Age, NT, PAPP-A and inhibin, 1st trimester, risk 1:100	1	40
65 Age, NT, PAPP-A and inhibin, 1st trimester, risk 1:250	1	40
66 Age, NT, PAPP-A and inhibin, 1st trimester, risk 1:400	1	40
67 Age, NT, PAPP-A and inhibin, 1st trimester, 5FPR	1	1110
68 Age, NT, PAPP-A and inhibin, 1st trimester, mixed cut-points	2	1150
69 Age, NT, PAPP-A and ADAM12, 1st trimester, 5FPR	2	1042
70 Age, NT, PAPP-A and ADAM12, 1st trimester, risk 1:250	1	691
71 Age, NT, free ßhCG and ADAM12, 1st trimester, 5FPR	1	351
72 Age, NT, AFP and free ßhCG, 1st trimester, risk 1:250	1	1656
73 Age, NT, AFP and free ßhCG, 1st trimester, 5FPR	1	1110
74 Age, NT, AFP and free ßhCG, 1st trimester, mixed cut-points	2	2766
75 Age, NT, AFP and PAPP-A, 1st trimester, 5FPR	1	1110
76 Age, NT, total hCG and PAPP-A, 1st trimester, 5FPR	1	1110
77 Age, NT, total hCG and inhibin, 1st trimester, 5FPR	1	1110
78 Age, NT, free ßhCG and inhibin, 1st trimester, 5FPR	1	1110
79 Age, NT, PAPP-A, free ßhCG, 1st trimester serum, ductus venosus pulsivity index, 5FPR	1	7250
80 Age, free ßhCG and PAPP-A, if risk 1:42-1:1000, NT, final 1:250 risk	1	10189
81 Age, NT, ductus, free ßhCG and PAPP-A, 1st trimester, risk 1:100	2	26986
82 Age, NT, ductus, free ßhCG and PAPP-A, 1st trimester, risk 1:250	2	10325
83 Age, NT, ductus, free ßhCG and PAPP-A, 1st trimester, 5FPR	2	10325
84 Age, NT, ductus, free ßhCG and PAPP-A, 1st trimester, mixed cut-points	3	30061
85 Age, NT, nasal bone, free ßhCG and PAPP-A, 1st trimester, risk 1:100	1	19736
86 Age, NT, nasal bone, free ßhCG and PAPP-A, 1st trimester, risk 1:300	1	1801
87 Age, NT, tricuspid blood flow, free ßhCG and PAPP-A, 1st trimester, risk 1:100	1	19736
88 Age, NT, fetal heart rate, free ßhCG and PAPP-A, 1st trimester, 5FPR	2	76385
89 Age, NT, fetal heart rate, nasal bone, free ßhCG and PAPP-A, 1st trimester, risk 1:200	1	19736
90 age, NT, fetal heart rate, ductus, free ßhCG and PAPP-A, 1st trimester, 5FPR	1	19614

Test	No. of studies	No. of participants
91 Age, NT, fetal heart rate, tricuspid blood flow, free ßhCG and PAPP-A,1st trimester, 5FPR	1	19736
92 Age, NT, AFP, free ßhCG and PAPP-A, 1st trimester, risk 1:250	1	5483
93 Age, NT, AFP, free ßhCG and PAPP-A, 1st trimester, 5FPR	2	1306
94 Age, NT, AFP, free ßhCG and PAPP-A, 1st trimester, mixed cut-points	3	6789
95 Age, NT, total hCG, inhibin and PAPP-A, 1st trimester, 5FPR	1	1110
96 Age, NT, PAPP-A, free ßhCG and PGH, 1st trimester, 5FPR	1	335
97 Age, NT, PAPP-A, free ßhCG and GHBP, 1st trimester, 5FPR	1	335
98 Age, NT, PAPP-A, free ßhCG and PIGF, 1st trimester, 5FPR	2	1443
99 Age, NT, PAPP-A, free ßhCG and total hCG, 1st trimester, 5FPR	1	998
100 Age, NT, PAPP-A, free ßhCG and PP13, 1st trimester, 5FPR	1	998
101 Age, NT, PAPP-A, free ßhCG and ADAM12, 1st trimester, 5FPR	4	2571
102 Age, NT, PAPP-A, free ßhCG and ADAM12, 1st trimester, risk 1:250	2	1222
103 Age, NT, PAPP-A, free ßhCG and ADAM12, 1st trimester, mixed cut-points	4	2571
104 Age, NT, free ßhCG, PAPP-A and inhibin, 1st trimester, risk 1:100	1	40
105 Age, NT, free ßhCG, PAPP-A and inhibin, 1st trimester, risk 1:250	1	40
106 Age, NT, free ßhCG, PAPP-A and inhibin, 1st trimester, risk 1:400	1	40
107 Age, NT, free ßhCG, PAPP-A and inhibin, 1st trimester, 5FPR	1	1110
108 Age, NT, PAPP-A, free ßhCG, ADAM12 and PlGH, 1st trimester, 5FPR	1	998
109 Age, NT, total hCG, inhibin, PAPP-A, AFP and uE3, 1st trimester, 5FPR	1	1110
110 Age, NT, free ßhCG, inhibin, PAPP-A, AFP and uE3,1st trimester, 5FPR	1	1110
111 Age, NT, PAPP-A, free ßhCG, ADAM12, total hCG and PlGF, 1st trimester, 5FPR	1	998
112 Age, NT, PAPP-A, free ßhCG, ADAM12, total hCG, PlGF and PP13, 1st trimester, 5FPR	1	998
113 NT, free ßhCG and PAPP-A, 1st trimester incidence rate 63.3%	1	6508
114 NT, PAPP-A, free ßhCG and maternal age - maternal age < 35 years	5	19057
115 NT, PAPP-A, free ßhCG and maternal age - maternal age ≥ 35 years	5	10980

Test 1. Aberrant right subclavian artery.

Test 2. Frontomaxillary facial angle >95 percentile.

Test 3. Presence of mitral gap.

Test 4. Maxillary bone length, 5% percentile.

Test 5. Tricuspid regurgitation.

Test 6. Iliac angle 90 degrees.

Test 7. Ductus venosus a-wave reversed.

Test 8. Ductus venosus pulsivity index > 95 percentile.

Test 9. Nasal bone, mixed cut-points.

Test 10. NT, 2.5 mm.

Test 11. NT, 3 mm.

Test 12. NT, 5FPR.

Test 13. NT, mixed cut-points.

Test 14. NT and age, risk 1:100.

Test 15. NT and age, risk 1:250.

Test 16. NT and age, risk 1:300.

Test 17. NT and age, 1FPR.

Test 18. NT and age, 3FPR.

Test 19. NT and age, 5FPR.

Test 20. NT and age, mixed cut-points.

Test 21. NT and nasal bone, Absent NB + NT ≥ 95th centile.

Test 22. Ductus and age, risk 1:250.

Test 23. Ductus and age, 5FPR.

Test 24. Ductus and age, mixed cut-points.

Test 25. Ductus, NT and age, risk 1:100.

Test 26. Ductus, NT and age, risk 1:250.

Test 27. Ductus, NT and age, 5FPR.

Test 28. Ductus, NT and age, mixed cut-points.

Test 29. Age and nasal bone, mixed cut-points.

Test 30. Age, NT and tricuspid blood flow, risk 1:100.

Test 31. Age, NT and nasal bone, risk 1:100.

Test 32. Age, NT and nasal bone, risk 1:300.

Test 33. Age, NT and nasal bone, mixed cut-points.

Test 34. Age, NT, nasal bone and ductus, risk NT>1:300 AND abnormal DV flow AND absent NB.

Test 35. Age, NT, nasal bone, free ßhCG and PAPP-A, 1st trimester, 5FPR.

Test 36. Age, NT, nasal bone, free ßhCG and PAPP-A, 1st trimester, mixed cut-points.

Test 37. Age, NT and free ßhCG, 1st trimester, 5FPR.

Test 38. Age, NT and free ßhCG, 1st trimester, risk 1:240.

Test 39. Age, NT and free ßhCG, 1st trimester, mixed cut-points.

Test 40. Age, NT and PAPP-A, 1st trimester, risk 1:100.

Test 41. Age, NT and PAPP-A, 1st trimester, risk 1:185.

Test 42. Age, NT and PAPP-A, 1st trimester, 5FPR.

Test 43. Age, NT and PAPP-A, 1st trimester, mixed cut-points.

Test 44. Age, NT and total hCG, 1st trimester, 5FPR.

Test 45. Age, NT and AFP, 1st trimester, 5FPR.

Test 46. Age, NT and ITA, 1st trimester, 5FPR.

Test 47. Age, NT and inhibin, 1st trimester, risk 1:100.

Test 48. Age, NT and inhibin, 1st trimester, risk 1:250.

Test 49. Age, NT and inhibin, 1st trimester, risk 1:400.

Test 50. Age, NT and inhibin, 1st trimester, 5FPR.

Test 51. Age, NT and inhibin, 1st trimester, mixed cut-points.

Test 52. Age, NT, PAPP-A and free ßhCG, 1st trimester, risk 1:100.

Test 53. Age, NT, PAPP-A and free ßhCG, 1st trimester, risk 1:150.

Test 54. Age, NT, PAPP-A and free ßhCG, 1st trimester, risk 1:200.

Test 55. Age, NT, PAPP-A and free ßhCG, 1st trimester, risk 1:220.

Test 56. Age, NT, PAPP-A and free ßhCG, 1st trimester, risk 1:250.

Test 57. Age, NT, PAPP-A and free ßhCG, 1st trimester, risk 1:300.

Test 58. Age, NT, PAPP-A and free ßhCG, 1st trimester, 1FPR.

Test 59. Age, NT, PAPP-A and free ßhCG, 1st trimester, 3FPR.

Test 60. Age, NT, PAPP-A and free ßhCG, 1st trimester, 5FPR.

Test 61. Age, NT, PAPP-A and free ßhCG, 1st trimester, mixed cut-points.

Test 62. Age, NT, PAPP-A and uE3, 1st trimester, 5FPR.

Test 63. Age, NT, PAPP-A and ITA, 1st trimester, 5FPR.

Test 64. Age, NT, PAPP-A and inhibin, 1st trimester, risk 1:100.

Test 65. Age, NT, PAPP-A and inhibin, 1st trimester, risk 1:250.

Test 66. Age, NT, PAPP-A and inhibin, 1st trimester, risk 1:400.

Test 67. Age, NT, PAPP-A and inhibin, 1st trimester, 5FPR.

Test 68. Age, NT, PAPP-A and inhibin, 1st trimester, mixed cut-points.

Test 69. Age, NT, PAPP-A and ADAM12, 1st trimester, 5FPR.

Test 70. Age, NT, PAPP-A and ADAM12, 1st trimester, risk 1:250.

Test 71. Age, NT, free ßhCG and ADAM12, 1st trimester, 5FPR.

Test 72. Age, NT, AFP and free ßhCG, 1st trimester, risk 1:250.

Test 73. Age, NT, AFP and free ßhCG, 1st trimester, 5FPR.

Test 74. Age, NT, AFP and free ßhCG, 1st trimester, mixed cut-points.

Test 75. Age, NT, AFP and PAPP-A, 1st trimester, 5FPR.

Test 76. Age, NT, total hCG and PAPP-A, 1st trimester, 5FPR.

Test 77. Age, NT, total hCG and inhibin, 1st trimester, 5FPR.

Test 78. Age, NT, free ßhCG and inhibin, 1st trimester, 5FPR.

Test 79. Age, NT, PAPP-A, free ßhCG, 1st trimester serum, ductus venosus pulsivity index, 5FPR.

Test 80. Age, free ßhCG and PAPP-A, if risk 1:42-1:1000, NT, final 1:250 risk.

Test 81. Age, NT, ductus, free ßhCG and PAPP-A, 1st trimester, risk 1:100.

Test 82. Age, NT, ductus, free ßhCG and PAPP-A, 1st trimester, risk 1:250.

Test 83. Age, NT, ductus, free ßhCG and PAPP-A, 1st trimester, 5FPR.

Test 84. Age, NT, ductus, free ßhCG and PAPP-A, 1st trimester, mixed cut-points.

Test 85. Age, NT, nasal bone, free ßhCG and PAPP-A, 1st trimester, risk 1:100.

Test 86. Age, NT, nasal bone, free ßhCG and PAPP-A, 1st trimester, risk 1:300.

Test 87. Age, NT, tricuspid blood flow, free ßhCG and PAPP-A, 1st trimester, risk 1:100.

Test 88. Age, NT, fetal heart rate, free ßhCG and PAPP-A, 1st trimester, 5FPR.

Test 89. Age, NT, fetal heart rate, nasal bone, free ßhCG and PAPP-A, 1st trimester, risk 1:200.

Test 90. age, NT, fetal heart rate, ductus, free ßhCG and PAPP-A, 1st trimester, 5FPR.

Test 91. Age, NT, fetal heart rate, tricuspid blood flow, free ßhCG and PAPP-A,1st trimester, 5FPR.

Test 92. Age, NT, AFP, free ßhCG and PAPP-A, 1st trimester, risk 1:250.

Test 93. Age, NT, AFP, free ßhCG and PAPP-A, 1st trimester, 5FPR.

Test 94. Age, NT, AFP, free ßhCG and PAPP-A, 1st trimester, mixed cut-points.

Test 95. Age, NT, total hCG, inhibin and PAPP-A, 1st trimester, 5FPR.

Test 96. Age, NT, PAPP-A, free ßhCG and PGH, 1st trimester, 5FPR.

Test 97. Age, NT, PAPP-A, free ßhCG and GHBP, 1st trimester, 5FPR.

Test 98. Age, NT, PAPP-A, free ßhCG and PIGF, 1st trimester, 5FPR.

Test 99. Age, NT, PAPP-A, free ßhCG and total hCG, 1st trimester, 5FPR.

Test 100. Age, NT, PAPP-A, free ßhCG and PP13, 1st trimester, 5FPR.

Test 101. Age, NT, PAPP-A, free ßhCG and ADAM12, 1st trimester, 5FPR.

Test 102. Age, NT, PAPP-A, free ßhCG and ADAM12, 1st trimester, risk 1:250.

Test 103. Age, NT, PAPP-A, free ßhCG and ADAM12, 1st trimester, mixed cut-points.

Test 104. Age, NT, free ßhCG, PAPP-A and inhibin, 1st trimester, risk 1:100.

Test 105. Age, NT, free ßhCG, PAPP-A and inhibin, 1st trimester, risk 1:250.

Test 106. Age, NT, free ßhCG, PAPP-A and inhibin, 1st trimester, risk 1:400.

Test 107. Age, NT, free ßhCG, PAPP-A and inhibin, 1st trimester, 5FPR.

Test 108. Age, NT, PAPP-A, free ßhCG, ADAM12 and PlGH, 1st trimester, 5FPR.

Test 109. Age, NT, total hCG, inhibin, PAPP-A, AFP and uE3, 1st trimester, 5FPR.

Test 110. Age, NT, free ßhCG, inhibin, PAPP-A, AFP and uE3,1st trimester, 5FPR.

Test 111. Age, NT, PAPP-A, free ßhCG, ADAM12, total hCG and PlGF, 1st trimester, 5FPR.

Test 112. Age, NT, PAPP-A, free ßhCG, ADAM12, total hCG, PlGF and PP13, 1st trimester, 5FPR.

Test 113. NT, free ßhCG and PAPP-A, 1st trimester incidence rate 63.3%.

Test 114. NT, PAPP-A, free ßhCG and maternal age - maternal age < 35 years.

Test 115. NT, PAPP-A, free ßhCG and maternal age - maternal age ≥ 35 years.

ADDITIONAL TABLES

Table 1. Direct (head-to-head) comparisons of the diagnostic accuracy of the 10 most evaluated first trimester ultrasound markers alone or in combination with first trimester serum tests

Ratio of DORs (95% CI); P value (Studies)	Nasal bone	NT	Nasal bone and age	Ductus and age	NT and age	NT, nasal bone and age	NT, free ßhCG and age	NT, PAPP-A and age	NT, PAPP-A, free ßhCG and age
NT	–								
Nasal bone and age	–	–							
Ductus and age	1.19 (0.12, 11.4); P = 0.84 (*K* = 1)	–	0.85 (0.21, 3.41); P = 0.76 (*K* = 1)						
NT and age	0.62 (0.13, 2.93); P = 0.50 (*K* = 2)	1.25 (0.90, 1.74); P = 0.17 (*K* = 3)	0.84 (0.48, 1.49); P = 0.52 (*K* = 3)	1.07 (0.51, 2.23); P = 0.85 (*K* = 3)					
NT, nasal bone and age	0.61 (0.12, 3.10); P = 0.50 (*K* = 2)	–	4.01 (1.51, 10.6); P = 0.01 (*K* = 2)	0.95 (0.23, 3.97); P = 0.93 (*K* = 1)	1.05 (0.70, 1.56); P = 0.82 (*K* = 5)				
NT, free ßhCG and age	–	2.15 (1.33, 3.50); P = 0.007 (*K* = 2)	–	–	1.47 (1.00, 2.15); P = 0.05 (*K* = 4)	–			
NT, PAPP-A and age	–	2.86 (1.73, 4.73); P = 0.001 (*K* = 2)	–	–	1.88 (1.27, 2.78); P = 0.004 (*K* = 4)	–	1.28 (0.84, 1.93); P = 0.23 (*K* = 4)		
NT, PAPP-A, free ßhCG and age	3.83 (0.89, 16.4); P = 0.07 (*K* = 2)	4.35 (2.00, 9.46); P = 0.015 (*K* = 4)	–	3.00 (0.42, 21.2); P = 0.19 (*K* = 1)	3.19 (2.19, 4.66); P < 0.0001 (*K* = 25)	1.23 (0.63, 2.40); P = 0.50 (*K* = 2)	2.06 (1.31, 3.22); P = 0.004 (*K* = 4)	1.61 (1.02, 2.55); P = 0.043 (*K* = 4)	

Table 1. Direct (head-to-head) comparisons of the diagnostic accuracy of the 10 most evaluated first trimester ultrasound markers alone or in combination with first trimester serum tests *(Continued)*

NT, PAPP-A, free ßhCG, ADAM 12 and age	–	–	–	–	–	–	–	–	0.87 (0.49, 1.52); P = 0.60 (*K* = 4)

– Indicates pairs of tests where there were no head-to head comparisons of the two tests in a study. 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.

Table 2. Indirect comparisons of the diagnostic accuracy of the 10 most evaluated first trimester ultrasound markers alone or in combination with first trimester serum tests

Ratio of DORs (95% CI); P value		**Nasal bone**	**NT**	**Nasal bone and age**	**Ductus and age**	**NT and age**	**NT, nasal bone and age**	**NT, free ßhCG and age**	**NT, PAPP-A and age**	**NT, PAPP-A, free ßhCG and age**
	DOR (95% CI)	132 (71, 245) *K* = 11	45 (31, 67) *K* = 13	40 (7, 224) *K* = 4	41 (18, 92) *K* = 5	46 (37, 57) *K* = 50	66 (24, 180) *K* = 5	65 (51, 84) *K* = 5	80 (59, 109) *K* = 5	133 (114, 155) *K* = 69
	Studies									
NT	45 (31, 67) *K* = 13	0.34 (0.16, 0.71); P = 0.006								
Nasal bone and age	40 (7, 224) *K* = 4	0.31 (0.05, 1.90); P = 0.18	0.90 (0.16, 5.05); P = 0.89							
Ductus and age	41 (18, 92) *K* = 5	0.31 (0.11, 0.87); P = 0.03	0.90 (0.37, 2.20); P = 0.80	1.00 (0.11, 9.34); P = 1.00						
NT and age	46 (37, 57) *K* = 50	0.35 (0.19, 0.66); P = 0.002	1.02 (0.66, 1.58); P = 0.92	1.14 (0.23, 5.61); P = 0.87	1.14 (0.52, 2.49); P = 0.74					
NT, nasal bone and age	66 (24, 180) *K* = 5	0.50 (0.14, 1.81); P = 0.26	1.47 (0.47, 4.58); P = 0.48	1.64 (0.12, 21.5); P = 0.62	1.64 (0.33, 8.08); P = 0.46	1.43 (0.52, 3.98); P = 0.48				

Table 2. Indirect comparisons of the diagnostic accuracy of the 10 most evaluated first trimester ultrasound markers alone or in combination with first trimester serum tests *(Continued)*

NT, free ßhCG and age	65 (51, 84) *K* = 5	0.49 (0.25, 0.98); P = 0.04	1.44 (0.89, 2.34); P = 0.12	1.61 (0.26, 10.1); P = 0.56	1.61 (0.65, 3.99); P = 0.26	1.41 (1.02, 1.96); P = 0.04	0.98 (0.30, 3.19); P = 0.98			
NT, PAPP-A and age	80 (59, 109) *K* = 5	0.61 (0.29, 1.25); P = 0.16	1.77 (1.05, 3.00); P = 0.04	1.98 (0.30, 13.1); P = 0.42	1.98 (0.76, 5.15); P = 0.14	1.73 (1.19, 2.53); P = 0.005	1.21 (0.35, 4.13); P = 0.73	1.23 (0.74, 2.05); P = 0.35		
NT, PAPP-A, free ßhCG and age	133 (114, 155) *K* = 69	1.00 (0.55, 1.84); P = 1.00	2.93 (1.96, 4.40); P < 0.0001	3.27 (0.68, 15.8); P = 0.14	3.27 (1.53, 7.00); P = 0.003	2.87 (2.21, 3.72); P < 0.0001	2.00 (0.73, 5.45); P = 0.17	2.03 (1.52, 2.72) P < 0.0001	1.65 (1.17, 2.34) P = 0.005	
NT, PAPP-A, free ßhCG, ADAM 12 and age	85 (58, 124) *K* = 4	0.64 (0.30, 1.37); P = 0.23	1.88 (1.07, 3.32); P = 0.03	2.10 (0.31, 14.1); P = 0.39	2.10 (0.78, 5.63); P = 0.12	1.84 (1.19, 2.84); P = 0.007	1.28 (0.37, 4.47); P = 0.65	1.30 (0.81, 2.09) P = 0.26	1.06 (0.61, 1.86) P = 0.81	0.64 (0.43, 0.96) P = 0.03

Indirect comparisons were made using all available data for each pair of tests. Ratios 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.

Table 3. Summary of study characteristics

Study	NT, PAPP-A, free ßhCG and age	Nasal bone	NT and age	NT	Maternal age (range) in years	Reference standard	Population	Study design	Study location
Acacio 2001				X	Mean 35.8 (21-45)	CVS biopsy, amniocentesis or blood or placenta used for fetal karyotyping	High-risk referral for invasive testing	Retrospective study of patient notes	South America
Audibert 2001			X		Mean 30.1, all < 38, 86% < 35, 14% ≥ 35	Prenatal karyotype conducted (in 7.6% of patients) depending on presence of risk > 125, high maternal age, parental anxiety, history of chromosomal defects or parental translocation or abnormal second trimester scan age	Routine screening	Prospective consecutive series	France

Table 3. Summary of study characteristics *(Continued)*

Babbur 2005			X	Median 37 (19-46)	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 hybridisation test in patients with risk > 1:30. No details given of any follow-up to birth	Women requesting screening (self-paying service) and women attending on account of previous pregnancy history of fetal abnormality	Prospective cohort	UK
Barrett 2008	X			Mean 34.9 for screen positives, 30.5 for screen negatives	Karyotyping or follow-up to birth	Routine screening	Cohort	Australia
Belics 2011				Mean 36.4 (15-46) for Down's cases, 29.8 (15-49) for unaffected pregnancies	Amniocentesis or CVS (85% of women) or follow-up to birth	High-risk referral for invasive testing	Cohort	Budapest
Benattar 1999		X		Mean 32 (16-46), 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	Routine screening	Prospective cohort	France
Bestwick 2010	X	X	X	Median 39 for Down's cases, 34 for unaffected pregnancies	Karyotyping or follow-up to birth	Routine screening	Retrospective cohort	UK
Biagiotti 1998	X	X		Unclear (maybe all ≥ 38)	Amniocentesis or CVS	High-risk referral for invasive testing	Case control	Italy

Table 3. Summary of study characteristics *(Continued)*

Boren-stein 2008			Median 35 (17-49)	CVS	High-risk referral for invasive testing	Prospective cohort	UK
Borrell 2005	X	X	Not reported	CVS (high-risk women) or follow-up to birth	Routine screening	Retrospective cohort	Spain
Borrell 2009	X		Mean 32	Karyotyping or follow-up to birth	Routine screening and high-risk referral	Prospective cohort	Spain
Brameld 2008	X		Median 31 (14-47), 20% ≥ 35	Karyotyping or follow-up to birth	Routine screening	Retrospective cohort	Australia
Brizot 2001		X	Median 28 (13-46), 19.4% ≥ 35	Antenatal karyotyping (5.9% of pregnancies: 62% of high-risk, 29% of medium-risk and 3% of the low-risk women). Follow-up to birth (85.3% of women)	Routine screening	Prospective cohort	Brazil
Centini 2005	X		≥ 35 (35-44)	Amniocentesis in women high risk on screening (16.2%). Follow-up at birth in women who were low risk on screening	High-risk patients undergoing routine screening	Retrospective cohort	Italy
Chasen 2003		X	Median 33 (IQR 31-36), 36.2% ≥ 35	Karyotyping or follow-up to birth in 96.1% of patients	Routine screening	Prospective consecutive cohort	USA
Chen 2009			Median 30 (20-44) for Down's cases, 32 (19-40) for controls	Karyotyping or follow-up to birth	Routine screening	Case control	China
Chris-tiansen 2005	X		Not reported	Karyotyping	Screening programmes for syphilis and Down's syndrome	Case control	Denmark

Table 3. Summary of study characteristics *(Continued)*

Christiansen 2009	X			Median 37.5 for Down's cases, 36.4 for controls	Karyotyping or follow-up to birth	Routine screening	Case control	Denmark
Christiansen 2010	X			Median 36 (25-44) for Down's cases, 29 (17-45) for controls	Karyotyping or follow-up to birth	Routine screening	Case control	Denmark
Cicero 2004a				Median 37 (16-48)	CVS	High-risk referral for invasive testing	Prospective cohort	USA
Cicero 2006		X		Median 35 (18-50)	CVS or amniocentesis (in high risk women) or follow-up to birth	Routine screening	Prospective cohort	UK
Cocciolone 2008 (first trimester screening cohort)	X			Median 31.3	Karyotyping or follow-up to birth	Routine screening	Cohort	Australia
Cowans 2009	X			Mean 38 (16-49) for Down's cases, 29 (13-56) for unaffected pregnancies	Karyotyping or follow-up to birth	Routine screening	Cohort	UK
Cowans 2010	X			Mean 37.0 (IQR 32.9-40.5) for Down's cases, 32.4 (IQR 29.0-35.9) for controls	Karyotyping or follow-up to birth	Routine screening	Case control	UK
Crossley 2002	X		X	Median 29.9, 15.4% ≥ 35	CVS (offered where women had high NT measurements), amniocentesis or follow-up to birth	Routine screening	Prospective cohort	UK

Table 3. Summary of study characteristics *(Continued)*

De Graaf 1999	X		X		Not reported	CVS and amniocentesis	High-risk referral for invasive testing	Case control	Netherlands
Ekelund 2008	X				Not reported	Karyotyping or follow-up to birth	Routine screening	Cohort	Denmark
Gasiorek-Wiens 2001			X		Median 33 (15-49), 36.1% > 35	CVS, amniocentesis or follow-up to birth	Routine screening	Prospective cohort	Germany, Switzerland and Austria
Gasiorek-Wiens 2010			X		Median 35.1 (13.2-46.7)	Karyotyping or follow-up to birth	Routine screening	Cohort	Germany
Go 2005	X				49% ≤ 35, 51% ≥ 36	Invasive testing or follow-up to birth	Routine screening	Retrospective cohort	Netherlands
Gyselaers 2005	X		X		Not reported	CVS, amniocentesis or follow-up to birth	Routine screening	Prospective cohort	Belgium
Habayeb 2010					Median 35.4 (18-49)	Karyotyping or follow-up to birth	Routine screening	Cohort	UK
Hadlow 2005*	X				Mean 30.7, 21.2% ≥ 35	CVS, amniocentesis or follow-up to birth	Routine screening	Prospective cohort	Australia
Hafner 1998*				X	Median 28 (15-49) 6.9% ≥ 35	Amniocentesis or CVS in patients with previous Down's pregnancy, > 35 years or with a positive biochemical test result. Other women underwent scan at 22 weeks and, if NT >2.5 mm special examination directed to examination of fetal heart. Follow-up to birth	Routine screening	Prospective cohort	Austria
Has 2008	X	X	X		Median 28.3 (17-45)	Karyotyping or follow-up to birth	Routine screening	Cohort	Turkey
Hewitt 1996				X	Median 37 (21-48)	CVS	High-risk referral for invasive testing	Prospective cohort	Australia

Table 3. Summary of study characteristics *(Continued)*

Hormans-dorfer 2011	X			Mean 31.1 (16-46), 22% ≥ 35	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	Germany
Huang 2010	X			Median 30 (15-47), mean 29.8 (SD 3.3)	Karyotyping or follow-up to birth	Routine screening	Cohort	Taiwan
Jaques 2007	X			Mean 33 (16-51), 18.5% ≥ 37	Karyotyping or follow-up to birth	Routine screening	Retrospec-tive cohort	Australia
Jaques 2010 FTS (first trimester screening)	X			Mean 16.3% ≥ 37	Karyotyping or follow-up to birth	Routine screening	Retrospec-tive cohort	Australia
Kagan 2010	X	X		Mean 35.4 (14.1-52.2)	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	UK
Kim 2006			X	Mean 29.9 (SD 3.3)	Amniocentesis or CVS in patients considered high risk (NT > 2.5, aged > 35 years, positive biochemical test result, history or chromoso-mal abnormality, fetal structural abnormality at ultrasound or other reason). Follow-up to birth	Routine screening	Retrospec-tive cohort	South Ko-rea
Koster 2011	X			Median 37 (IQR 36-39)	Karyotyping or follow-up to birth	Routine screening	Case control	Nether-lands
Kozlows-ki 2007 GC (Gynae-cologists' practices)	X	X		Median 32 (15-48), 26.4% ≥ 35	Karyotyping or follow-up to birth	Routine screening	Cohort	Germany
Kozlows-ki 2007 PC (Prenatal centre)	X	X		Median 34 (14-46), 43.2% ≥ 35	Karyotyping or follow-up to birth	Routine screening	Cohort	Germany

Table 3. Summary of study characteristics *(Continued)*

Krantz 2000*	X		X	34.7% ≥ 35	Not reported	Routine screening	Prospective cohort	USA
Kublickas 2009	X			51% ≥ 35	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	Sweden
Kuc 2010	X			Not reported	Karyotyping or follow-up to birth	Routine screening	Case control	Nether-lands
Lam 2002			X	Mean 30.5 (19% ≥ 35) for unaf-fected pregnan-cies	Women considered high risk of-fered CVS (0.7%) or amniocentesis (11.8%). Follow-up to birth	Routine screening	Prospective cohort	Hong Kong
Leung 2009	X	X		Median 32 (IQR 30-35), 27.4% ≥ 35	Amniocentesis or follow-up to birth	Routine screening	Prospective cohort	China
MacRae 2008			X	Not reported	Karyotyping or follow-up to birth	Routine screening	Retrospec-tive cohort	UK
Maiz 2007				Median 35 (17-49)	CVS	High-risk re-ferral for inva-sive testing	Prospective cohort	UK
Maiz 2009				Median 34.5 (14.1-50.1)	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	UK
Malone 2004		X		Mean 30.1 (16-47), 22.1% ≥ 35	Amniocentesis (in women consid-ered high risk, n = 510) or follow-up to birth	Routine screening	Prospective cohort	USA
Malone 2005	X			21.6% ≥ 35	Amniocentesis offered to women with positive results from any screening test. Follow-up to birth	Routine screening	Prospective cohort	USA
Marchini 2010*	X			Median 31.3 (18-45), 19.7% ≥ 35	Karyotyping or follow-up to birth	Routine screening	Retrospec-tive cohort	Italy

Table 3. Summary of study characteristics *(Continued)*

Marsis 2004			X	Mean 37.8 (35-43)	Amniocentesis (unclear in which patients this was conducted) or follow-up to birth	Screening of patients ≥ 35 years of age	Prospective cohort	Indonesia
Marsk 2006	X	X		Mean 38.5 (SD 4.0) for Down's cases, 35.5 (SD 4.0) for controls	Not reported	Routine screening	Case control	Sweden
Matias 1998				Median 35 (17-46)	Fetal karyotyping. In cases where NT above 95th percentile or abnormal ductus venousus flow, follow-up scan conducted at 14-16 weeks	High-risk referral for invasive testing	Prospective cohort	UK and Portugal
Matias 2001				Median 35 (17-46)	Fetal karyotyping. In cases where NT above 95th percentile or abnormal ductus venousus flow, follow-up scan conducted at 14-16 weeks	High-risk referral for invasive testing	Prospective cohort	Portugal
Mavrides 2002		X		Median 35 (15-42)	CVS or follow-up	High-risk referral for invasive testing	Prospective cohort	UK
Maxwell 2011 FTS (first trimester screening cohort)	X			Median 31 (14-48), 24.3% ≥ 35	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	Australia
Maymon 2005				Mean 33.7 (SD 4.9) for Down's cases, 30.3 (SD 4.5) for controls	Amniocentesis (recommended for women with higher risk on first or second trimester testing) or follow-up to birth	Routine screening	Case control	Israel
Maymon 2008	X	X		Not reported	Karyotyping or follow-up to birth	Routine screening	Case control	USA
Merz 2011	X			Not reported	Karyotyping or follow-up to birth	Routine screening	Retrospective cohort	Germany

Table 3. Summary of study characteristics *(Continued)*

Michailidis 2001				X	Mean 30.1 (13-50), 21.1% ≥ 35, 11.9% ≥ 37	Karyotyping in women considered at risk due to index test results, age or family history or those with considerable anxiety (632 women, 8.5%) or follow-up to birth	Routine screening	Prospective cohort	UK
Molina 2010 high risk (High-risk cohort)		X			Mean 32.7 (16.7-47.5)	CVS	High-risk referral for invasive testing	Cohort	Spain
Molina 2010 screening (Screening cohort)	X				Not reported	Karyotyping or follow-up to birth	Routine screening	Cohort	Spain
Monni 2005			X		Median 32 (14-49)	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	Italy
Montalvo 2005	X				Mean 31.1 (14-49), 25.9% ≥35	Invasive testing offered to women considered high risk from screening results or follow-up to birth	Routine screening	Prospective cohort	Spain
Moon 2007		X			Mean 35.5 (SD 4.8) for Down's cases, 31.7 (SD 3.4) for unaffected pregnancies	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	Korea
Muller 2003	X		X		Not reported	Invasive testing (offered to women with high NT measurement) or follow-up to birth	Routine screening	Retrospective cohort	France
Nicolaides 1992				X	Median 38 (22-47)	Fetal karyotyping by amniocentesis (52%) or CVS (48%)	High-risk referral for invasive testing	Prospective cohort	UK
Nicolaides 2005	X				Median 31 (13-49)	Amniocentesis or CVS (patients considered high risk based on screening). First trimester presence/absence of nasal bone, pres-	Routine screening	Prospective cohort	UK

Table 3. Summary of study characteristics *(Continued)*

					ence/absence of tricuspid regurgitation or normal/abnormal Doppler studies (patients of intermediate risk on first trimester screening and did not undergo CVS or amniocentesis. With the addition of information from these tests, if adjusted risk was high, CVS was performed). Follow-up to birth			
Niemimaa 2001	X		X		17.5% ≥ 35	Invasive testing (patients considered high risk based on NT screening) or follow-up to birth.	Routine screening	Prospective cohort	Finland
Noble 1995					Median 34 (15-47), 47% ≥ 35	Karyotyping performed (27% of women) due to increased NT (14%), advanced maternal age (10%), previous chromosomally abnormal child (0.5%) or parental anxiety (2%). Ultrasound examination at 20 weeks (65% of patients). Follow-up to birth (9% of women)	Routine screening in a high risk population	Prospective cohort	UK
O'Callaghan 2000			X		Median 32	CVS, amniocentesis or neonatal karyotyping or follow-up to birth	Routine screening	Prospective cohort	Australia
O'Leary 2006	X		X		Median 31 (14-47), 20% ≥ 35	CVS or amniocentesis (women assessed to be high risk on screening) or follow-up to birth	Routine screening	Prospective cohort	Australia
Okun 2008 FTS (first trimester screening cohort)	X				Mean 34	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	Canada
Orlandi 1997	X		X		Range 15 to 46, 35% ≥ 35	Not reported	Routine screening	Prospective cohort	Italy
Orlandi 2003		X			Median 31.7 (SD 4.0) for Down's cases, 36.5 (SD 4.1) for unaf-	CVS or amniocentesis (women considered high risk on screening on the basis of NT and biochemical results, but not on nasal bone	Routine screening (2 centres) or in referred pa-	Prospective cohort	Italy and Netherlands

Table 3. Summary of study characteristics *(Continued)*

					fected pregnancies	screening, or if requested due to age or anxiety) or follow-up to birth	tients (1 centre)		
Orlandi 2005		X			Median 30.5 (SD 8.2)	Not reported	Routine screening	Retrospective cohort	Italy
Otaño 2002		X			Median 36 (19-44)	CVS	High-risk referral for invasive testing	Prospective cohort	Argentina
Pajkrt 1998			X		Mean 31.4 (SD 5.7), 24% ≥ 35	Prenatal karyotyping offered to patients considered high risk or maternal anxiety (conducted in 24%) or follow-up to birth	Routine screening	Prospective cohort	Netherlands
Pajkrt 1998a				X	Mean 37.6 (22-46)	Prenatal karyotyping	High-risk referral for invasive testing	Consecutive cohort	Netherlands
Palomaki 2007 FTS (first trimester screening cohort)					Mean 32.3 (SD 4.6)	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	Canada
Perni 2006	X				Median 33.0 (IQR 31.0-36.0)	CVS or amniocentesis. Cytogenetic testing in cases of miscarriage. Follow-up to birth.	Routine screening	Retrospective cohort	USA
Prefumo 2005			X		Median 37 (19-46)	CVS	High-risk referral for invasive testing	Prospective cohort	UK
Prefumo 2006			X		Mean 31.4 (14.5-50.2)	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	UK
Ramos-Corpas 2006		X			Mean 30.1 (15-46) (SD 5.37), 18% ≥ 35	Invasive testing offered to patients considered high risk at screening (> 1:300) or follow-up to birth	Routine screening	Prospective cohort	Spain
Rissanen 2007	X				29.5, 17.7% ≥35	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	Finland

Table 3. Summary of study characteristics *(Continued)*

Rozenberg 2002		X		Median 30.5 (18-37)	Amniocentesis offered to patients with NT >3mm or serum marker risk was > 1:250, or follow-up to birth	Routine screening	Prospective cohort	France
Rozenberg 2007	X			Mean 30.9 (SD 4.5)	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	Canada
Sahota 2010	X	X		Median 33.1, 30.1% ≥ 35	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	China
Salomon 2010	X			Median 30.7 (18.0-46.3)	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	France
Santiago 2007	X	X		Mean 30.6 (14-46)	Karyotyping or follow-up to birth	Routine screening	Retrospective cohort	Spain
Sau 2001		X		Mean 28 (SD 5)	Invasive testing (women with high risk on screening) or follow-up to birth	Routine screening	Prospective cohort	UK
Schaelike 2009	X	X		31.0% ≥35	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	Germany
Schielen 2006*	X			Median 36.5 (18-47)	Invasive testing or follow-up to birth	Routine screening	Retrospective cohort	Netherlands
Schuchter 2001		X		Mean 28 (15-46), 10.7% ≥ 35	CVS (offered to patients with first trimester NT > 3.5 mm), amniocentesis (offered to patients with first trimester NT 2.5-3.4 mm, high risk on second trimester serum testing (> 1:250) and those > 35 years) or follow-up to birth	Routine screening	Retrospective cohort	Austria
Schuchter 2002	X		X	13% > 35	CVS and amniocentesis (offered to patients with increased risk (> 1:400) at first trimester screening. CVS recommended when NT > 3.5 or when women did not want to wait until the 15th week for amniocentesis), or follow-up to birth	Routine screening	Prospective cohort	Austria

Table 3. Summary of study characteristics *(Continued)*

Schwarzler 1999			X		Mean 29.4 (16-47)	Invasive testing (women considered high risk on screening) or follow-up to birth	Routine screening	Prospective consecutive cohort	UK
Scott 2004	X		X		Median 32 (15-44), 29% ≥ 35	Invasive testing or follow-up to birth	Routine screening	Prospective cohort	Australia
Sepulveda 2007		X	X		Median 33 (14-47), 35.4% ≥ 35	CVS, amniocentesis, cordocentesis or follow-up to birth	Routine screening	Prospective cohort	Chile
Snijders 1998			X		Median 31 (14-49)	CVS and amniocentesis (9.6% of women) or follow-up to birth	Routine screening	Prospective cohort	UK
Sorensen 2011	X				Median 34 (23-44) for Down's cases; mean 30.4 (16-45), 16.5% ≥ 35 for unaffected pregnancies	Karyotyping or follow-up to birth	Routine screening	Retrospective cohort	Denmark
Spencer 1999	X		X	X	Median 38 (19-46) for Down's cases, 36 (15-47) for controls	Invasive testing (high-risk women) or follow-up to birth	Routine screening	Case control	UK
Spencer 2002					Median 36 (20-44) for Down's cases, 30 (16-41) for controls	Not reported	Routine screening	Case control	UK
Spencer 2008	X				Median 35.8 for Down's cases, 29.3 for controls	Karyotyping or follow-up to birth	Routine screening	Case control	Denmark
Stenhouse 2004	X				Median 32 (14-45), 27% ≥ 35	Invasive testing offered to women with screening risk of > 1:250 or follow-up to birth	Routine screening	Prospective cohort	UK

Table 3. Summary of study characteristics *(Continued)*

Strah 2008		X		Median 28.6 (15-42)	Karyotyping or follow-up to birth	Routine screening	Cohort	Slovenia
Theodor-opoulos 1998		X		Median 29 (16-48), 7.8% ≥ 37	CVS or amniocentesis or follow-up to birth. Unclear reference standard in cases of intrauterine death, miscarriages and terminations.	Routine screening	Prospective cohort	Greece
Thila-ganathan 1999		X		Mean 29 (15-45)	CVS (offered to patients considered high risk on screening) or follow-up to birth	Routine screening	Prospective cohort	UK
Timmer-man 2010				Mean 34.5 (19-45)	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	Nether-lands
Torring 2010	X			Mean 35 for Down's cases, 31 for controls	Karyotyping or follow-up to birth	Routine screening	Case control	Denmark
Vadiveloo 2009	X			Median 33.1, 36.9% ≥ 35	Karyotyping or follow-up to birth	Routine screening	Retrospec-tive cohort	UK
Valinen 2007	X			Mean 29.6, 18.6% ≥ 35	Karyotyping or follow-up to birth	Routine screening	Retrospec-tive cohort	Finland
Viora 2003				Median 32 (18-47)	CVS or follow-up to birth	Routine screening	Prospective cohort	Italy
Wald 2003	X	X	X	Not reported	Invasive testing (following second trimester screening) or follow-up to birth	Routine screening	Case control	UK and Austria
Wapner 2003*	X	X		Mean 35 (SD 4.6), 50% ≥ 35	Invasive testing. Miscarriage with cytogenetic testing. Follow-up to birth	Routine screening	Prospective cohort	USA
Wax 2009	X			Mean 36.7 (SD 3.2)	Karyotyping or follow-up to birth	Routine screening	Retrospec-tive cohort	USA
Wojde-mann 2005	X	X		Mean 29, 10.8% ≥ 35	Invasive testing (in cases of increased risk) or follow-up to birth	Referrals for screening	Prospective cohort	Denmark

Table 3. Summary of study characteristics *(Continued)*

Wortelboer 2009	X		Median 34.9 (15-48)	Karyotyping or follow-up to birth	Routine screening	Cohort	Netherlands
Wright 2008		X	Median 35.2 (16-52)	Karyotyping or follow-up to birth	Routine screening	Cohort	UK
Wright 2010	X		Median 31.9 (IQR 27.7-35.8)	Karyotyping or follow-up to birth	Routine screening	Cohort	UK, Denmark and Cyprus
Zoppi 2001		X	Median 33 (14-48)	Amniocentesis, CVS or follow-up to birth	Routine screening	Prospective cohort	Italy

*The study provided data for the subset of women with maternal age of 35 or more.
X indicates that the test was evaluated in the study.
CVS = chorionic villus sampling; IQR = interquartile range; SD = standard deviation.

Table 4. Investigation of the effect of type of population

Correction made for missing false negatives in studies with delayed verification of test negatives	NT			NT and maternal age			NT, PAPP-A, free ßhCG and maternal age		
	Ratio of DORs (95% CI); P value	Sensitivity at 5% FPR (95% CI) (studies)		Ratio of DORs (95% CI); P value	Sensitivity at 5% FPR (95% CI) (studies)		Ratio of DORs (95% CI); P value	Sensitivity at 5% FPR (95% CI) (studies)	
		Screening (n = 9)	High risk (n = 4)		Screening (n = 46)	High risk (n = 4)		Screening (n = 66)	High risk (n =3)
No FN correction	0.68 (0.26, 1.77); P = 0.40	73 (62, 81)	64 (45, 80)	0.34 (0.17, 0.69); P = 0.003	72 (68, 76)	47 (31, 63)	0.41 (0.16, 1.00); P = 0.05	88 (86, 89)	74 (54, 88)
FN increased +10%	0.69 (0.27, 1.78); P = 0.40	70 (59, 79)	62 (42, 78)	0.40 (0.20, 0.82); P = 0.01	69 (64, 73)	47 (31, 64)	0.48 (0.19, 1.20); P = 0.11	86 (84, 87)	74 (53, 88)
FN increased +20%	0.74 (0.29, 1.92); P = 0.50	69 (57, 78)	62 (42, 78)	0.43 (0.21, 0.89); P = 0.02	67 (63, 71)	47 (31, 64)	0.51 (0.20, 1.28); P = 0.15	85 (83, 87)	74 (54, 88)
FN increased +30%	0.81 (0.31, 2.09); P = 0.63	67 (55, 76)	62 (42, 78)	0.46 (0.22, 0.97); P = 0.04	66 (61, 70)	47 (30, 64)	0.55 (0.22, 1.38); P = 0.20	84 (82, 86)	74 (54, 88)

Table 4. Investigation of the effect of type of population *(Continued)*

FN increased +40%	0.76 (0.29, 2.02); P = 0.55	66 (53, 76)	59 (39, 77)	0.50 (0.24, 1.02); P = 0.06	64 (60, 68)	47 (31, 64)	0.59 (0.24, 1.48); P = 0.26	83 (81, 85)	74 (54, 88)
FN increased +50%	0.81 (0.30, 2.15): P = 0.65	64 (52, 75)	59 (39, 77)	0.52 (0.25, 1.08); P = 0.08	63 (58, 67)	47 (30, 64)	0.62 (0.25, 1.56); P = 0.31	82 (80, 84)	74 (54, 88)

DOR = diagnostic odds ratio

APPENDICES

Appendix 1. Search strategy

Database: Ovid MEDLINE

---

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 41 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

---

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. "unconjugated oestriol"
26. "unconjugated estriol"
27. afp
28. "alpha fetoprotein*"
29. alphafetoprotein*
30. " b hcg"
31. "human chorionic gonadotrophin"
32. "papp a"
33. "pregnancy associated plasma protein"
34. "nuchal translucency"
35. foetal
36. fetal
37. foetus
38. fetus
39. prenatal*
40. antenatal*
41. pregnan*
42. trimester*
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*))))))

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.

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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 90% specificity. In addition, a requirement that a minimum of 10 studies for a single test was required before subgroup analysis was undertaken. Consequently, several potential sources of heterogeneity were not investigated due to lack of data. To investigate the impact of multifetal pregnancies, we excluded studies in a sensitivity analysis to determine the effect on our estimates of test accuracy. This was done because data were limited for meta-regression analyses.

We intended to conduct sensitivity analyses on the analysis investigating the effect of maternal age on test sensitivity. This was not possible due to limited data. Instead we performed the sensitivity analyses when comparing high-risk populations with routine screening populations. This comparison was considered a proxy for the effect of maternal age because the main indication for referral for invasive testing was often increased risk due to advanced maternal age. Due to lack of information, we were unable to consider the impact of age standardisation and improvements in technology on the estimates of test performance.

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 (this review)
- First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening (in press).

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.

INDEX TERMS

Medical Subject Headings (MeSH)

*Ultrasonography, Prenatal; Biomarkers [blood]; Chorionic Gonadotropin [blood]; Chorionic Gonadotropin, beta Subunit, Human [blood]; Down Syndrome [*blood] [*diagnosis] [diagnostic imaging]; False Positive Reactions; Maternal Age; Nasal Bone [diagnostic imaging]; Pregnancy Trimester, First [*blood]; Pregnancy-Associated Plasma Protein-A [analysis]; Sensitivity and Specificity

MeSH check words

Female; Humans; Pregnancy