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Urine tests for Down's syndrome screening (Review)

Allred SK, Guo B, Takwoingi Y, Pennant M, Wisniewski S, Deeks JJ, Neilson JP, Alfirevic Z

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Urine tests for Down's syndrome screening (Review)

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[Diagnostic Test Accuracy Review]

Urine tests for Down's syndrome screening

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ABSTRACT

Background

Down's syndrome occurs when a person has three copies of chromosome 21, or the specific area of chromosome 21 implicated in causing Down's syndrome, rather than two. It is the commonest congenital cause of mental disability 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. The risk of a Down's syndrome affected pregnancy increases with advancing maternal age.

Noninvasive 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 and second trimester urine markers for the detection of Down's syndrome.

Search methods

We carried out a sensitive and comprehensive literature search of MEDLINE (1980 to 25 August 2011), EMBASE (1980 to 25 August 2011), BIOSIS via EDINA (1985 to 25 August 2011), CINAHL via OVID (1982 to 25 August 2011), The Database of Abstracts of Reviews of Effectiveness (*The Cochrane Library* 2011, Issue 7), MEDION (25 August 2011), The Database of Systematic Reviews and Meta-Analyses in Laboratory Medicine (25 August 2011), The National Research Register (archived 2007), Health Services Research Projects in Progress database (25 August 2011). We studied reference lists and published review articles.

Selection criteria

Studies evaluating tests of maternal urine in women up to 24 weeks of gestation for Down's syndrome, compared with a reference standard, either chromosomal verification or macroscopic postnatal inspection.

Data collection and analysis

We extracted data as test positive or 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 (Quality Assessment of Diagnostic Accuracy Studies) criteria. We used hierarchical summary ROC (receiver operating characteristic) meta-analytical methods to analyse test performance and compare test accuracy. We performed analysis of studies allowing direct comparison between tests. We investigated the impact of maternal age on test performance in subgroup analyses.

Main results

We included 19 studies involving 18,013 pregnancies (including 527 with Down's syndrome). Studies were generally of high quality, although differential verification was common with invasive testing of only high-risk pregnancies. Twenty-four test combinations were evaluated formed from combinations of the following seven different markers with and without maternal age: AFP (alpha-fetoprotein), ITA (invasive trophoblast antigen), β -core fragment, free β hCG (beta human chorionic gonadotrophin), total hCG, oestriol, gonadotropin peptide and various marker ratios. The strategies evaluated included three double tests and seven single tests in combination with maternal age, and one triple test, two double tests and 11 single tests without maternal age. Twelve of the 19 studies only evaluated the performance of a single test strategy while the remaining seven evaluated at least two test strategies. Two marker combinations were evaluated in more than four studies; second trimester β -core fragment (six studies), and second trimester β -core fragment with maternal age (five studies).

In direct test comparisons, for a 5% false positive rate (FPR), the diagnostic accuracy of the double marker second trimester β -core fragment and oestriol with maternal age test combination was significantly better (ratio of diagnostic odds ratio (RDOR): 2.2 (95% confidence interval (CI) 1.1 to 4.5), $P = 0.02$) (summary sensitivity of 73% (CI 57 to 85) at a cut-point of 5% FPR) than that of the single marker test strategy of second trimester β -core fragment and maternal age (summary sensitivity of 56% (CI 45 to 66) at a cut-point of 5% FPR), but was not significantly better (RDOR: 1.5 (0.8 to 2.8), $P = 0.21$) than that of the second trimester β -core fragment to oestriol ratio and maternal age test strategy (summary sensitivity of 71% (CI 51 to 86) at a cut-point of 5% FPR).

Authors' conclusions

Tests involving second trimester β -core fragment and oestriol with maternal age are significantly more sensitive than the single marker second trimester β -core fragment and maternal age, however, there were few studies. There is a paucity of evidence available to support the use of urine testing for Down's syndrome screening in clinical practice where alternatives are available.

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 or CVS to confirm a diagnosis of Down's.

What we did

The aim of this review was to find out which of the urine screening tests done during the first 24 weeks of pregnancy are the most accurate at predicting the risk of a pregnancy being affected by Down's. We looked at seven different urine markers that can be used alone, in ratios or in combination, taken before 24 weeks' gestation, thus creating 24 screening tests for Down's. We found 19 studies, involving 18,013 pregnancies of which 527 had pregnancies affected by Down's.

What we found

For the first 24 weeks of pregnancy, the evidence does not support the use of urine tests for Down's syndrome screening. The amount of evidence is limited. These tests are not offered in routine clinical practice.

Other important information to consider

Urine tests for Down's syndrome screening (Review)

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The urine tests themselves have no adverse effects for the woman. 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

Summary of findings 1. Performance of urine tests with or without maternal age

Review Question	What is the accuracy of urine based markers for screening for Down's syndrome?
Population	Pregnant women at less than 24 weeks' gestation confirmed by ultrasound, who had not undergone previous testing for Down's syndrome. Most studies were undertaken in women identified to be high risk based on maternal age
Settings	All settings
Numbers of studies, pregnancies and Down's syndrome cases	19 studies (reported in 29 publications) involving 18,013 pregnancies of which 527 were Down's syndrome pregnancies.
Index tests	Risk scores computed using maternal age and first and second trimester urine markers for AFP; ITA; β -core fragment; free β hCG; total hCG; oestriol (also termed as uE3); gonadotropin peptide.
Reference standards	Chromosomal verification (amniocentesis and CVS undertaken during pregnancy, and postnatal karyotyping) and postnatal macroscopic inspection.
Study limitations	Seven studies only used selective chromosomal verification during pregnancy, and were at risk of under-ascertainment of Down's syndrome cases due loss of the pregnancy to miscarriage between the serum test and the reference standard.

Test	Studies	Women (Cases)	Sensitivity* (95% CI)	Specificity* (95% CI)	Threshold
Test without maternal age					
<i>Single tests</i>					
First trimester free β hCG	1	516 (86)	5 (1 to 11)	95 (92 to 97)	5% FPR
First trimester β -core fragment	1	516 (86)	10 (5 to 19)	95 (92 to 97)	5% FPR
First trimester ITA	2	579 (94)	15 (2 to 62)	95	5% FPR
First trimester total hCG	1	516 (86)	17 (10 to 27)	95 (92 to 97)	5% FPR
Second trimester oestriol	2	1472 (47)	23 (8 to 49)	95	5% FPR

Second trimester total hCG	1	390 (65)	31 (20 to 43)	95 (92 to 97)	5% FPR
Second trimester free β hCG	3	1517 (107)	32 (12 to 63)	95	5% FPR
Second trimester β -core fragment	6	9613 (193)	41 (20 to 66)	95	5% FPR
Second trimester ITA	3	2748 (131)	43 (35 to 51)	95	5% FPR
Second trimester β -core fragment to oestriol ratio	2	1649 (35)	74 (58 to 86)	95	5% FPR
Second trimester gonadotropin test	1	105 (14)	93 (66 to 100)	95 (88 to 98)	1:384 risk
Double tests					
Second trimester AFP and ITA	1	524 (24)	79 (58 to 93)	95 (93 to 97)	5% FPR
Second trimester β -core fragment and oestriol	1	315 (24)	83 (63 to 95)	95 (92 to 97)	5% FPR
Triple tests					
Second trimester AFP, uE3 and ITA	1	524 (24)	79 (58 to 93)	95 (93 to 97)	5% FPR
Test with maternal age					
Single tests					
Second trimester oestriol	1	474 (69)	49 (37 to 62)	95 (92 to 97)	5% FPR
Second trimester β -core fragment	5	3419 (155)	56 (45 to 66)	95	5% FPR
Second trimester free β hCG	2	879 (98)	57 (47 to 67)	95	5% FPR
Second trimester free β hCG to oestriol ratio	1	474 (69)	64 (51 to 75)	95 (92 to 97)	5% FPR
Second trimester β -core fragment to free β hCG	1	474 (69)	67 (54 to 78)	95 (92 to 97)	5% FPR
Second trimester ITA	1	1016 (23)	70 (47 to 87)	95 (93 to 96)	5% FPR
Second trimester β -core fragment to oestriol ratio	3	2088 (105)	71 (51 to 86)	95	5% FPR
Double tests					
Second trimester oestriol and free β hCG	1	474 (69)	68 (56 to 79)	95 (92 to 97)	5% FPR

Second trimester β -core fragment and oestriol	2	1631 (92)	73 (57 to 85)	95	5% FPR
Second trimester AFP and β -core fragment to oestriol ratio	1	356 (10)	90 (55 to 100)	95 (93 to 97)	1:58 risk

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

AFP: alpha-fetoprotein; **β hCG:** beta human chorionic gonadotrophin; **CI:** confidence interval; **CVS:** chorionic villus sampling; **FPR:** false positive rate; **hCG:** beta human chorionic gonadotrophin; **ITA:** invasive trophoblast antigen; **uE3:** unconjugated oestriol

BACKGROUND

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

Target condition being diagnosed

Down's syndrome

Down's syndrome affects approximately one in 800 live-born babies (Cuckle 1987a). It results from a person having three, rather than two, copies of chromosome 21—or the specific area of chromosome 21 implicated in causing Down's syndrome, as a result of trisomy 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. Noninvasive 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 et al 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 et al 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 β sub-unit 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 1995a; 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, β -core fragment, free β hCG and total hCG (Cole 1999a). There is controversy about their value (Wald 2003a).

Screening and parental choice

Antenatal screening is used for several reasons (Alfirevic 2004), but the most important is to enable parental choice regarding pregnancy management and outcome. Before a woman and her partner opt to have a screening test, they need to be fully informed about the risks, benefits and possible consequences of such a test. This includes the choices they may have to face should the result show that the woman has a high risk of carrying a baby with Down's syndrome and the implications of both false positive and false negative screening tests. They need to be informed of the risk of a miscarriage due to invasive diagnostic testing, and the possibility that a miscarried fetus may be chromosomally normal. If, following invasive diagnostic testing, the fetus is shown to have Down's syndrome, further decisions need to be made about continuation or termination of the pregnancy, the possibility of adoption and finally, preparation for parenthood. Equally, if a woman has a test that shows she is at a low risk of carrying a fetus with Down's

syndrome, it does not necessarily mean that the baby will be born with a normal chromosomal make up. This possibility can only be excluded by an invasive diagnostic test (Alfirevic 2003). The 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 urine screening tests used in the first and second trimester of pregnancy (up to 24 weeks' gestation) comprised of the following individual markers; AFP; invasive trophoblast antigen (ITA) (also known as hyperglycosylated hCG); β -core fragment; free β hCG; total hCG; uE3 (oestriol); gonadotropin peptide; and various marker ratios. 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 (Vaklenti 1968). Amniocentesis is an invasive procedure that 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). Recent developments in the use of cell-free fetal DNA detection in maternal serum are paving the way for noninvasive diagnosis of Down's syndrome and other trisomies, however these tests were not used as reference standards in any of the studies examined.

Many different screening tests are available and offered to pregnant women, and these tests are the subject of additional Cochrane reviews published (Aldred 2012) or currently in preparation, and other published reviews. Tests being assessed in other Cochrane reviews include first trimester serum tests; second trimester serum tests; first trimester ultrasound markers; tests that combine serum and ultrasound markers; and tests that combine markers from

the first trimester with markers from the second trimester. 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 (Aldred 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 give comparative results between the best tests in the different categories. This review is written with a 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 that 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 and second trimester urine 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 of women with a low risk (normal) screening test result who subsequently had a baby unaffected by Down's syndrome (specificity). We grouped our analyses to focus on investigating the value of adding increasing numbers of markers (comparing single, dual, triple and quadruple tests).

Investigation of sources of heterogeneity

We planned to investigate whether a uniform screening test is suitable for all women, or whether different screening methods are more applicable to different groups, defined by advanced maternal age, ethnic groups and aspects of the pregnancy and medical history such as multiple pregnancy, diabetes and family history

of Down's syndrome. We also considered whether there existed evidence of overestimation of test accuracy in studies evaluating risk equations in the derivation sample rather than in a separate validation sample.

METHODS

Criteria for considering studies for this review

Types of studies

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

Participants

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

Index tests

The following index tests were examined; AFP; ITA; β -core fragment; free β hCG; total hCG; oestriol (also termed as uE3); gonadotropin peptide and various marker ratios and combinations of these markers combined with maternal age. Combinations without maternal age were not included in the test comparisons (Table 1; Table 2), however, information on such test combinations is provided.

We looked at comparisons of tests in isolation and in various combinations. These included single (one marker), double (two markers), triple (three markers), test strategies, all maternal age-adjusted.

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

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

Databases searched included;

- MEDLINE via OVID (1980 to 25 August 2011)
- EMBASE via Dialog Datastar (1980 to 25 August 2011)
- BIOSIS via EDINA (1985 to 25 August 2011)
- CINAHL via OVID (1982 to 25 August 2011)
- The Database of Abstracts of Reviews of Effectiveness (*The Cochrane Library* 2011, Issue 7)
- MEDION (25 August 2011)
- The Database of Systematic Reviews and Meta-Analyses in Laboratory Medicine (www.ifcc.org/) (25 August 2011)
- The National Research Register (archived 2007)
- 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 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 we 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. We obtained full-text versions of studies identified as being potentially relevant and two review authors independently assessed these 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

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

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

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

Assessment of methodological quality

We used a modified version of the QUADAS tool ([Whiting 2003](#)), a quality assessment tool for use in systematic reviews of diagnostic accuracy studies, to assess the methodological quality of included studies. We anticipated that a key methodological issue would be the potential for bias arising from the differential use of invasive testing and follow-up for the reference standard according to index test results, bias arising due to higher loss to miscarriage in false negatives than true negatives. We chose to code this issue as originating from differential verification in the QUADAS tool: we are aware that it could also be coded under delay in obtaining

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

QUADAS criteria included the following 10 questions.

1. Was the spectrum of women representative of the women who will receive the test in practice? (Criteria met if the sample was selected from a wide range of childbearing ages, or selected from a specified 'high-risk' group such as over 35s, family history of Down's syndrome, multiple pregnancy or diabetes mellitus, provided all affected and unaffected fetuses included that could be tested at the time point when the screening test would be applied; criteria not met if the sample taken from a select or unrepresentative group of women (i.e. private practice), was an atypical screening population or recruited at a later time point when selection could be affected by selective fetal loss).
2. Is the reference standard likely to correctly classify the target condition? (Amniocentesis, CVS, 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 that reported a common threshold to estimate average

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

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

Comparisons between tests

We made comparisons between tests, first by utilising all available studies, selecting one threshold from each study to estimate a SROC curve without restricting to a common threshold. The threshold was chosen for each study according to the following order of preference: a) the risk threshold closest to one in 250; b) a multiples of the median (MoM) or presence/absence threshold; c) the performance closest to a 5% FPR or 95th percentile. The 5% FPR was chosen as a cut-off point as this is the cut-off most commonly reported in the literature. The analysis that used all available studies was performed by including the 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 model 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 we obtained associated confidence intervals 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. We used a separate model to make each pair-wise comparison. We assessed comparisons between tests by using likelihood ratio tests to test if the differences in accuracy were statistically significant or not. We expressed the differences as ratios of diagnostic odds ratios and 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

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

Sensitivity analyses

In many of the included studies, mothers with pregnancies identified as high risk for Down's syndrome by the urine testing were offered immediate definitive testing by amniocentesis, whereas the remainder 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 urine testing (the false negative diagnoses). Testing and detection of miscarriages is impractical in many situations, and no clear data are available on the magnitude of these miscarriage rates.

To account for the possible bias introduced by such a mechanism, we planned to perform sensitivity analyses by increasing the percentage of false negatives in studies where delayed verification in test negatives occurred ([Mol 1999](#)). We planned to incrementally increase the percentage from 10% to 50%, the final value representing a scenario where a third of more Down's pregnancies than normal pregnancies were likely to miscarry, thought to be higher than the likely value. We intended to conduct the sensitivity analyses on the analysis investigating the effect of maternal age on test sensitivity.

RESULTS

Results of the search

The search for the whole suite of reviews identified a total of 15,394 papers, once the results from each bibliographic database were combined and duplicates were removed. After screening out obviously 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. We included a total of 19 studies (reported in 29 publications) in this review of urine tests, involving 18,013 pregnancies, of which 527 were Down's syndrome pregnancies.

A total of 24 different test strategies or combinations, at one or more thresholds, were evaluated in the 19 studies. These tests were produced from combinations of seven different urine tests (and their ratios) with and without maternal age: AFP; ITA; β -core fragment; free β hCG; total hCG; oestriol; gonadotropin peptide and various marker ratios. Strategies evaluated included three double tests and seven single tests in combination with maternal age, and one triple test, two double tests and 11 single tests without maternal age. Twelve of the 19 studies only evaluated the

performance of a single test strategy while the remaining seven evaluated at least two test strategies.

The following combinations evaluated included four or more studies.

1. Second trimester β -core fragment (six studies; 9615 women with 193 affected Down's pregnancies)
2. Second trimester β -core fragment and maternal age (five studies; 3419 women with 155 Down's pregnancies)

Methodological quality of included studies

We judged the studies to be of high methodological quality in most categories ([Figure 1](#)). Due to the nature of testing for

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

Figure 1. Methodological quality summary: review authors' judgements about each methodological quality item for each included study.

	Representative spectrum?	Acceptable reference standard?	Partial verification avoided?	Differential verification avoided?	Incorporation avoided?	Reference standard results blinded?	Index test results blinded?	Relevant clinical information?	Uninterpretable results reported?	Withdrawals explained?
Bahado-Singh 1998	+	+	+	+	+	-	+	+	-	-
Bahado-Singh 1998b	+	+	+	+	+	-	+	+	-	-
Bahado-Singh 1999	+	+	+	+	+	-	+	+	-	-
Bahado-Singh 1999a	+	+	+	+	+	-	+	+	-	-
Bahado-Singh 2000	+	+	+	+	+	-	+	+	-	-
Bahado-Singh 2000a	+	+	+	+	+	+	?	+	-	-
Canick 1995	+	+	-	-	+	+	?	+	-	-
Cole 1997a	+	+	+	+	+	+	+	+	-	-
Cole 1997b	+	+	+	+	+	+	?	+	-	-
Cole 1999b	+	+	+	-	+	+	+	+	-	-
Cuckle 1995b	+	+	+	-	+	+	+	+	-	-
Cuckle 1999	+	+	+	-	+	+	?	+	-	-
Cuckle 1999a	+	+	?	-	+	-	+	+	-	-
Hsu 1999	+	+	+	+	+	+	?	+	-	-
Isozaki 1997	+	+	+	+	+	+	+	+	-	-
Palomaki 2004a	+	+	+	+	+	+	+	+	-	-
Spencer 1996	+	+	+	-	+	+	+	+	-	-
Wald 2003	+	+	?	-	+	-	?	+	+	-
Weinans 2000	+	+	+	+	+	+	+	+	-	-

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

Findings

1) Second trimester β -core fragment

Results for this single test were derived from six studies (Cole 1999b; Cuckle 1995b; Cuckle 1999a; Isozaki 1997; Spencer 1996; Wald 2003), and included 9615 women in whom 193 pregnancies were known to be affected by Down's syndrome. Two studies (Cole 1999b; Cuckle 1999a) contributed over 7000 pregnancies to the data. Six studies (Cole 1999b; Cuckle 1995b; Cuckle 1999a; Isozaki 1997; Spencer 1996; Wald 2003) presented data for a cut-point of 5% FPR and the estimated sensitivity was 41% (95% confidence interval (CI) 20 to 66).

2) Second trimester β -core fragment and maternal age

Results for this single test were derived from five studies (Bahado-Singh 1999; Bahado-Singh 1999a; Cole 1999b; Hsu 1999; Spencer 1996), and included 3419 women in whom 155 pregnancies were known to be affected by Down's syndrome. Cole 1999b contributed over 1000 pregnancies to the data. The studies presented data at a cut-point of 5% FPR and the summary sensitivity was 56% (95% CI 45 to 66).

3) Other test combinations

Of the 22 test combinations evaluated in three or fewer studies, nine test combinations demonstrated estimated sensitivities of more than 70% and estimated specificities of more than 90%. Six of these were evaluated in single studies (see [Summary of findings 1](#)), and the following three test combinations were evaluated in two or more studies.

1. **Second trimester β -core fragment to oestriol ratio** evaluated in two studies (Cole 1997b; Cole 1999b), with a summary sensitivity of 74% (95% CI 58 to 86) at a cut-point of 5% FPR.
2. **Second trimester β -core fragment to oestriol ratio and maternal age** evaluated in three studies (Bahado-Singh 1999; Cole 1999b; Hsu 1999), with a summary sensitivity of 71% (95% CI 51 to 86) at a cut-point of 5% FPR.
3. **Second trimester β -core fragment, oestriol and maternal age** evaluated in two studies (Cole 1999b; Hsu 1999), with a summary sensitivity of 73% (95% CI 57 to 85) at a cut-point of 5% FPR.

Comparative analyses of the five selected test strategies

For each test we obtained the detection rate (sensitivity) for a fixed FPR (1-specificity), a metric which is commonly used in Down's syndrome screening to describe test performance. We chose to estimate detection rates at a 5% FPR in common with much of the literature. [Figure 2](#) shows point estimates of the detection rate (and their 95% CIs) at a 5% FPR based on all available data for the five test strategies; the test strategies are ordered according to decreasing detection rates. The plot shows that all five test strategies have detection rates between 56% and 90%. The combination of second trimester AFP and β -core fragment to oestriol ratio with maternal age showed the highest detection rate with an estimated detection rate of 90% (CI 55 to 100), based on data from one study with 10 affected cases out of a total of 356 pregnancies. The worst performing strategy was the combination of β -core fragment to oestriol ratio and maternal age, with an estimated detection rate of 56% (CI 45 to 66), based on data from five studies with 155 affected cases out of a total of 3419 pregnancies.

Figure 2. Detection rates (% sensitivity) at a 5% false positive rate for the five most evaluated or best performing test strategies. The estimates are shown with 95% confidence intervals. The test strategies are ordered on the plot according to decreasing detection rate. The number of studies, cases and women included for each test strategy are shown on the horizontal axis.

Table 1 shows pair-wise direct comparisons (head-to-head) where studies were available. Such comparisons are regarded as providing the strongest evidence as they are unconfounded. The table shows the ratio of diagnostic odds ratio (RDOR) with 95% CI and P values for each test combination, the number of studies (K) for which data were available. The table shows that the diagnostic accuracy of the double marker combination of second trimester β -core fragment and oestriol with maternal age was significantly better (RDOR 2.2 (95% CI 1.1 to 4.5); $P = 0.02$) than the single marker second trimester β -core fragment and maternal age test strategy but was not significantly better (RDOR 1.5 (95% CI 0.8 to 2.8); $P = 0.21$) than that of the second trimester β -core fragment to oestriol ratio and maternal age test strategy. However, the comparisons in

this table were based on two or three studies and are unlikely to be powered to detect differences in detection rates.

Table 2 shows the same comparisons made using all available data (as used to create Figure 2). Results are in agreement with the direct comparisons, and in addition, showed no significant differences between any of the other pair of tests for which direct comparisons were not available. However, these comparisons are potentially confounded by differences between the studies, and the evidence is limited.

Investigation of heterogeneity and sensitivity analyses

None of the tests was evaluated by 10 or more studies and so we were unable to investigate the effect of maternal age or any other potential source of heterogeneity. The planned sensitivity analyses, looking at differential verification and any resultant bias, were also not possible.

DISCUSSION

Summary of main results

The systematic review found 19 studies evaluating urinary markers for Down's syndrome screening. Very few studies provided unconfounded comparisons of test strategies by applying and comparing several strategies using the same urine sample; the majority of studies only evaluating a single test combination. A summary of results for the 24 strategies is given in [Summary of findings 1](#).

The following key findings were noted.

1. There is evidence from direct comparison to support the use of multiple marker urine tests in combination with age for screening - the double marker combination of second trimester β -core fragment and oestriol with maternal age test strategy was significantly better (ratio of diagnostic odds ratio (RDOR) 2.2 (95% CI 1.1 to 4.5); $P = 0.02$) than the single marker second trimester β -core fragment and maternal age. This is reflected in the indirect comparison of the two tests.
2. There was little evidence that urine markers are of value in screening for Down's syndrome. Marker combinations evaluated by more than three studies showed low detection rates for a 5% false positive rate (FPR). More promising markers were investigated in fewer than three studies.
3. In indirect comparisons, with the exception of the difference in accuracy between the single marker second trimester β -core fragment and maternal age test and the double marker combination of second trimester β -core fragment and oestriol with maternal age, there was no significant difference in the detection rates between tests, however, the number of included studies was small.

Strengths and weaknesses of the review

This is the first comprehensive systematic review of urine tests for Down's syndrome screening. We examined papers from around the world, covering a wide cross-section of women in varying populations. We contacted authors to verify data where necessary to give as complete a picture as possible while trying to avoid replication of data.

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

1. There were many different cut-points used to define pregnancies as high or low risk for Down's syndrome. This means that direct comparison is more difficult than if all studies used the same cut-point to dichotomise their populations.
2. There were many different risk equations and software applications in use for combination of multiple markers, which were often not described in the papers. This means that risks

may be calculated by different formulae, and they may not be directly comparable for this reason.

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/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. Very few studies make direct comparisons between tests, making it difficult to detect if there is a real difference between tests (i.e. how different tests perform in the same population). There are differences in populations, with assay medians being affected, for example, by race. It is not certain whether it is appropriate to make comparisons between populations which are inherently different.
5. We were unable to perform any of the subgroup analyses that we had originally intended to, as the data simply were not available. The vast majority of papers looking at pregnancies conceived by in vitro fertilisation (IVF), affected by diabetes, multiple gestation or a family history of Down's syndrome involved unaffected pregnancies only.

Applicability of findings to the review question

When planning a 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 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 the harms of testing, information about miscarriage rates and uptake of definitive testing as the data were not often available. 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 a single urine sample, differences in accuracy may lead to differences in the use of definitive testing and its consequent adverse outcomes.

AUTHORS' CONCLUSIONS

Implications for practice

Urine testing for Down's syndrome is not commonly used, with serum and ultrasound testing being widely clinically available. We would not recommend the introduction of urine testing for Down's syndrome screening on the basis of the review findings, or that urine testing should replace serum or ultrasound testing where it is available. There is a paucity of evidence available to support the use of urine testing in clinical practice where alternatives are available.

Implications for research

Further evaluation of urine tests is required before definitive recommendations can be made about their use in clinical practice. Future studies should ensure that adequate sample sizes are

recruited, and make comparisons of several alternative test combinations on the same urine samples. Such direct comparisons minimise confounding and allow a clear focus on testing the incremental benefit of increasingly complex and expensive testing strategies. The reporting of test accuracy studies can be improved by adhering to the STARD reporting guideline [Bossuyt 2003](#). Three key aspects 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.

For the purposes of meta-analysis and to allow for comparisons to be made between different tests and combinations, we 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]

Bahado-Singh 1998

Clinical features and settings	High-risk referral for invasive testing.
Participants	511 participants. USA. August 1996 to January 1997. Singleton pregnancies. Pregnant women. Mean age 37.1 years (SD 2.8 years). 15-24 weeks' gestation.
Study design	Prospective cohort study.

Bahado-Singh 1998 (Continued)

Target condition and reference standard(s)	Down's syndrome: 18 cases. Reference standard: amniocentesis.
Index and comparator tests	Mid-trimester urine β -core fragment testing (monoclonal antibody B210 assay, 2-step sandwich method, standardised for creatinine).
Follow-up	100% karyotyping.
Aim of study	To ascertain the screening efficiency of a new mid-trimester Down's syndrome detection protocol that combines maternal urine testing and ultrasonographic examination.
Notes	Amniocentesis was being conducted on the basis of maternal age. Women who have amniocentesis just due to abnormal screening results were excluded from the study.

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.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women underwent 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	Urine testing was conducted blind from the results of amniocentesis.
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.

Bahado-Singh 1998b

Clinical features and settings	High-risk referral for invasive testing.
Participants	356 participants: 10 cases and 346 controls. USA. Dates not reported. Singleton pregnancies. Pregnant women. 14-24 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 10 cases. Reference standard: amniocentesis.
Index and comparator tests	Second trimester urine β -core fragment testing (monoclonal antibody B210 assay, 2-step sandwich method, standardised for creatinine). Second trimester serum AFP. Risk cut points of 1/10, 1/20, 1/30, 1/58, 1/270, 1/526.
Follow-up	100% karyotyping.
Aim of study	To determine Down's syndrome screening efficiency of a new protocol that combines maternal serum AFP and beta core fragment/total oestriol ratio.
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.
Partial verification avoided? All tests	Yes	All women underwent a reference standard.
Differential verification avoided? All tests	Yes	All women underwent 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.

Bahado-Singh 1998b (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.

Bahado-Singh 1999

Clinical features and settings	High-risk referral for invasive testing.	
Participants	457 participants. USA. August 1996 - June 1997. Pregnant women. Mean age 37.1 years. Singleton pregnancies. 15-24 weeks' gestation.	
Study design	Prospective cohort study.	
Target condition and reference standard(s)	Down's syndrome: 13 cases. Reference standard: amniocentesis.	
Index and comparator tests	Maternal age. Urinary β core fragment (monoclonal antibody B210 assay, 2-step sandwich method, standardised for creatinine). Urinary beta core fragment/total urinary oestriol ratio.	
Follow-up	100% karyotyping.	
Aim of study	To evaluate Down's syndrome screening efficiency of a new algorithm of multiple urinary biochemical and ultrasound markers.	
Notes		

Table of Methodological Quality

Item	Authors' judgement	Description
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Urine tests for Down's syndrome screening (Review)

Bahado-Singh 1999 (Continued)

Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference stan- dard? All tests	Yes	Amniocentesis.
Partial verification avoid- ed? 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? 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 informa- tion? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results re- ported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

Bahado-Singh 1999a

Clinical features and set- tings	High-risk referral for invasive testing.
Participants	926 participants. USA. November 1995 - March 1999. Pregnant women. Singleton pregnancies. 15-24 weeks' gestation. Euploid/Down's karyotype only.
Study design	Prospective cohort study.

Bahado-Singh 1999a (Continued)

Target condition and reference standard(s)	Down's syndrome: 21 cases. Reference standard: amniocentesis.
Index and comparator tests	Maternal age. Second trimester urinary β core fragment (Spot specimens of urine - 2-step sandwich assay B120 monoclonal antibody). Second trimester serum AFP. Frozen serum samples tested for second trimester uE3 and free β hCG (details of serum testing methods not given).
Follow-up	100% karyotyping.
Aim of study	To compare Down's syndrome screening efficiency of elevated maternal urine level of beta core fragment with that of a traditional serum triple test.

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.
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?	No	No details of withdrawals given.

Urine tests for Down's syndrome screening (Review)

Bahado-Singh 1999a (Continued)

All tests

Bahado-Singh 2000

Clinical features and settings	High-risk referral for invasive testing.
Participants	1016 participants. USA. May 1995 - June 1998. Singleton pregnancies. Pregnant women. Mean age 37.1 years (19.3-46 years). 14-24 weeks' gestation. Euploid or Down's pregnancies only.
Study design	Prospective cohort study.
Target condition and reference standard(s)	Down's syndrome: 23 cases. Reference standard: amniocentesis.
Index and comparator tests	Second trimester urinary hyperglycosylated hCG (Specific monoclonal antibody developed. 2-step enzyme immunometric assay standardised for creatinine levels).
Follow-up	100% karyotyping.
Aim of study	To evaluate the measurement of levels of urine hyperglycosylated hCG in conjunction with ultrasound biometry for Down's syndrome risk prediction in an at risk group.
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.
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.

Urine tests for Down's syndrome screening (Review)

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Bahado-Singh 2000 (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.

Bahado-Singh 2000a

Clinical features and settings	High-risk referral for invasive testing.
Participants	<p>524 participants.</p> <p>USA - single hospital.</p> <p>August 1995 - April 1999.</p> <p>Singleton pregnancies.</p> <p>Pregnant women.</p> <p>Mean age 36.6 years (SD 5.3 years) in those with Down's detected and 37.0 years (SD 3.4 years) in those with euploid pregnancies.</p> <p>14-22 weeks' gestation.</p>
Study design	Prospective cohort study.
Target condition and reference standard(s)	<p>Down's syndrome: 24 cases.</p> <p>Reference standard: amniocentesis.</p>
Index and comparator tests	<p>Maternal age.</p> <p>Second trimester serum hCG (IMX total β-hCG kit, Abbott Laboratories), uE3 (DSL-1400 Ultra-sensitive unconjugated Estriol Radioimmunoassay kit) and AFP (IMX AFP kit, Abbott Laboratories).</p> <p>Second trimester urinary beta core fragment (Spot specimens of urine - 2-step sandwich assay B120 monoclonal antibody).</p> <p>Frozen samples tested for second trimester urinary hyperglycosylated hCG (Specific monoclonal antibody developed. 2-step enzyme immunometric assay standardised for creatinine levels).</p>

Bahado-Singh 2000a (Continued)

Follow-up	100% karyotyping.
Aim of study	To compare the concentration of hyperglycosylated human chorionic gonadotropin with serum triple screen for second trimester Down's syndrome detection.
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.
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	Yes	Reference standard interpreted without knowledge of index test results.
Index test results blinded? All tests	Unclear	Unclear if index test interpreted with 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.

Canick 1995

Clinical features and settings	Referral for termination of pregnancy, amniocentesis or routine examination.
Participants	105 participants: 14 cases and 91 controls. USA.

Canick 1995 (Continued)

	Dates not reported.
	Singleton pregnancies.
	Pregnant women.
	15-21 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 14 cases. Reference standard: karyotyping on termination of pregnancy or amniocentesis.
Index and comparator tests	Maternal age. Frozen samples tested for: second trimester urinary gonadotropin peptide (Triton UGP EIA assay, Alameda); second trimester serum hCG (MAIAclone hCG assay, Serono-Baker Diagnostics, Allentown).
Follow-up	No details given for any follow-up to birth. Reported that the fetal karyotype of control samples was not always known but assumed that none were aneuploid pregnancies.
Aim of study	To assess whether urinary gonadotropin peptide is better than serum hCG as a second trimester screening marker.
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening 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	No	Not all women received 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	Yes	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.

Canick 1995 (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.

Cole 1997a

Clinical features and settings	High-risk referral for invasive testing.	
Participants	722 participants. USA – single hospital. August 1995 - May 1996. Pregnant women. Singleton pregnancy. 12-24 weeks' gestation.	
Study design	Cohort study.	
Target condition and reference standard(s)	Down's syndrome: 13 cases. Reference standard: amniocentesis.	
Index and comparator tests	Second trimester urinary hCG free beta subunit (Immunoassay with autoantibody FBT11).	
Follow-up	100% karyotyping.	
Aim of study	To evaluate use of second trimester urinary free beta-subunit for Down's syndrome screening.	
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.
Partial verification avoided? All tests	Yes	All women had a reference standard.

Urine tests for Down's syndrome screening (Review)

Cole 1997a (Continued)

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	Yes	Reference standard interpreted without 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.

Cole 1997b

Clinical features and settings	High-risk referral for invasive testing.
Participants	492 participants. USA – single hospital. August 1995 - May 1996. Pregnant women. Singleton pregnancy. 12-24 weeks' gestation.
Study design	Prospective cohort study.
Target condition and reference standard(s)	Down's syndrome: 12 cases. Reference standard: amniocentesis.
Index and comparator tests	Second trimester urinary hCG free beta subunit (B210 2-step sandwich assay). Second trimester urinary total oestriol (radioimmunoassay, kit from Diagnostics Products Corporation, Los Angeles).
Follow-up	100% karyotyping.
Aim of study	To evaluate use of urinary free beta core fragment combined with urinary total oestriol for Down's syndrome screening.

Urine tests for Down's syndrome screening (Review)

Cole 1997b (Continued)

Notes

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference stan- dard? All tests	Yes	Amniocentesis.
Partial verification avoid- ed? 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	Yes	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 re- sults.
Relevant clinical informa- tion? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results re- ported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

Cole 1999b

Clinical features and set- tings	High-risk referral for invasive testing.
Participants	1157 participants. USA – 3 hospitals. May 1995 - March 1998. Pregnant women. Singleton pregnancy.

Cole 1999b (Continued)

11-22 weeks' gestation.

Study design	Prospective cohort study.
Target condition and reference standard(s)	Down's syndrome: 23 cases. Reference standards: amniocentesis or CVS.
Index and comparator tests	Urinary hCG beta-core subunit (B210 2-step sandwich assay). Urinary total oestriol (radioimmunoassay, kit by Diagnostic Products Corporation, Los Angeles).
Follow-up	100% karyotyping.
Aim of study	To evaluate use of urinary free beta-subunit for Down's syndrome screening.
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 CVS or amniocentesis depending on their stage of pregnancy.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results.
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results.
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

Cuckle 1995b

Clinical features and settings	High-risk referral for invasive testing and testing for bacterial analysis.
Participants	315 participants. UK. Dates not specified. Pregnant women: 24 cases undergoing invasive testing and 294 controls undergoing testing for bacterial analysis 11-23 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 24 cases. Reference standards: amniocentesis or CVS for cases and follow-up for controls.
Index and comparator tests	Urinary beta core fragment (Modified radioimmunoassay method). Urinary total oestrogen (continuous flow reaction based on the Kuber method).
Follow-up	No details given of methods of follow-up.
Aim of study	To evaluate the use of multiple urinary markers rather than serum in order to screen 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	Karyotyping or follow-up to birth.
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	Yes	Reference standard interpreted without knowledge of index test results.

Cuckle 1995b (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.

Cuckle 1999

Clinical features and settings	High-risk referral for invasive testing and routine screening.	
Participants	349 participants: 45 cases and 304 controls. UK. Dates not specified. Pregnant women. 14-19 weeks' gestation.	
Study design	Retrospective case-control study.	
Target condition and reference standard(s)	Down's syndrome: 45 cases. Reference standard: amniocentesis, CVS or follow-up to birth.	
Index and comparator tests	Frozen samples tested for urinary hyperglycosylated hCG (Immunoassays by 'Cole' method corrected for creatinine levels using Jaffes method).	
Follow-up	Details of follow-up not reported.	
Aim of study	To determine the distribution of hyperglycosylated hCG levels in pregnancies with 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	Amniocentesis, CVS or follow-up.
Partial verification avoided?	Yes	All women had a reference standard.

Urine tests for Down's syndrome screening (Review)

Cuckle 1999 (Continued)

All tests

Differential verification avoided? All tests	No	Women had different reference standards.
Incorporation avoided? All tests	Yes	Index tests did not form part of the reference standard.
Reference standard results blinded? All tests	Yes	Reference standard conducted before the index test.
Index test results blinded? All tests	Unclear	Index test conducted after the reference standard and no evidence of blinding.
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

Cuckle 1999a

Clinical features and settings	High-risk referral for invasive testing and routine screening.
Participants	6730 participants. USA, UK and other European countries –multicentre study. Dates not reported. Pregnant women. 14-19 weeks' gestation.
Study design	Prospective cohort study.
Target condition and reference standard(s)	Down's syndrome: 39 cases. Reference standard: amniocentesis, CVS or postnatal examination.
Index and comparator tests	Maternal urine beta core hCG (Chiron manual assay).
Follow-up	Methods of follow-up not reported.
Aim of study	A prospective evaluation of urine beta core hCG for Down's syndrome.
Notes	

Cuckle 1999a (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	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	No	Women had different reference standards.
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 conducted without knowledge of the reference standard.
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.

Hsu 1999

Clinical features and settings	High-risk referral for invasive testing.
Participants	474 participants: 69 cases and 405 controls. Taiwan and UK. Dates not specified. Pregnant women. Median age cases 36.0 years (21-44 years), controls 34.5 years (23-43 years). 14-26 weeks' gestation.

Hsu 1999 (Continued)

Study design	Retrospective case-control study.
Target condition and reference standard(s)	Down's syndrome: 69 cases. Reference standard: amniocentesis.
Index and comparator tests	Maternal age. Urinary beta core fragment (UGP) (UGF-EIA Toa kit). Urinary free beta hCG (CIS immunoradiometric assay). Urinary total oestriol (Orthoclinical diagnostics oestriol (total) II radioimmunoassay kit). All adjusted for creatinine concentration. Modelled to standardised population for England and Wales 1991-1994. Cases from Taiwan.
Follow-up	100% karyotyping.
Aim of study	To investigate levels of urinary beta core fragment, free beta hCG and total oestriol in a new large set of Down's syndrome pregnancies.
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.
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	Yes	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.

Hsu 1999 (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.

Isozaki 1997

Clinical features and settings	High-risk referral for invasive testing.	
Participants	726 participants. USA - single centre. August 1995 – May 1996. Pregnant women. Mean age 35.4 years (SD 4.0 years) in mothers of Down's syndrome babies and 37 years (SD 4.3 years) in mothers of healthy babies. Singleton pregnancies. 12-24 weeks' gestation.	
Study design	Prospective cohort study.	
Target condition and reference standard(s)	Down's syndrome: 13 cases. Reference standard: amniocentesis.	
Index and comparator tests	Urinary beta core fragment (B210 monoclonal antibody, 2-step sandwich assay).	
Follow-up	100% karyotyping.	
Aim of study	To present data for prospectively collected samples of urinary beta core fragment for Down's syndrome screening.	
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.
Partial verification avoided? All tests	Yes	All women had a reference standard.

Urine tests for Down's syndrome screening (Review)

Isozaki 1997 (Continued)

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	Yes	Reference standard interpreted without 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.

Palomaki 2004a

Clinical features and settings	High-risk referral for invasive testing.
Participants	2,055 participants. USA – multicentre study. January 2001 - January 2003. Pregnant women with mean age 38.9 years. 15-20 weeks' gestation.
Study design	Prospective cohort study.
Target condition and reference standard(s)	Down's syndrome: 28 cases. Reference standard: amniocentesis.
Index and comparator tests	Urinary invasive trophoblastic antigen (ITA) (B207 (detection) and B152 (capture) anti-hCG monoclonal antibodies).
Follow-up	100% karyotyping.
Aim of study	To evaluate ITA as a potential marker for Down's syndrome in the second trimester of pregnancy.
Notes	Clean catch of random urine provided. Sent same day at 4 degrees Celcius on an ice pack. Aliquoted into 1 mL plastic tubes. 1 urine aliquot shipped to lab for testing. Rest stored at -70 degrees Celcius. Most

Palomaki 2004a (Continued)

samples assayed within 24 hours of reaching lab and all within 48 hours. Anti-ITA antibody produced. Sample corrected for creatinine levels.

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk population as done in practice.
Acceptable reference standard? All tests	Yes	Amniocentesis.
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	Yes	Reference standard interpreted without 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 1996

Clinical features and settings	High-risk referral for invasive testing.
Participants	429 participants: 29 cases and 400 controls. UK. Date not specified. Pregnant women. Singleton pregnancies.

Urine tests for Down's syndrome screening (Review)

Spencer 1996 (Continued)

14-24 (cases) and 9-22 (controls) weeks' gestation.

Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 29 cases. Reference standards: amniocentesis or CVS.
Index and comparator tests	Urine free beta hCG (CIS immunoradiometric assay). Urinary beta core fragment (Ciba Corning diagnostics UGP enzyme immunoassay).
Follow-up	100% karyotyping.
Aim of study	To evaluate whether free beta hCG is elevated in the urine of pregnancies affected by Down's syndrome and investigate whether urine free beta hCG may be used as possible screening markers.
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	Yes	Reference standard interpreted without 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.

Urine tests for Down's syndrome screening (Review)

Wald 2003

Clinical features and settings	Routine screening.
Participants	606 participants: 101 cases, 505 controls matched for gestation, duration of storage and centre. UK and Austria - multicentre trial. September 1996 - April 2000. Pregnant women. 9-13 and 14-20 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 101 cases. Reference standards: invasive testing (following second trimester screening) or follow-up to birth.
Index and comparator tests	First trimester NT (midsagittal section, optimal magnification of thickness of translucent space between inner skin surface and fascia covering cervical spine (white black interface (outer) - black white interface (inner), 41 models of ultrasound machine, 20 minutes allotted scanning time). First and second trimester serum AFP, hCG, uE3, PAPP-A, free beta hCG (time resolved fluoroimmunoassay, AutoDELFIA). First and second trimester inhibin A (Sandwich enzyme-linked immunosorbent assay, Oxford Bio-innovation). First and second trimester urinary beta core fragment, total hCG, ITA and free beta hCG (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 cytogenetic 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 women in the total cohort did not have a documented outcome of pregnancy. Unclear if any of these women were included in this 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? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard?	Yes	Karyotyping or follow-up to birth.

Urine tests for Down's syndrome screening (Review)

Wald 2003 (Continued)

All tests

Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	No	Women received 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	Unclear	Serum testing conducted after reference standard and unclear if 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.

Weinans 2000

Clinical features and settings	High-risk referral for invasive testing.
Participants	63 participants: 8 cases and 55 controls matched for gestational and maternal age, maternal weight, duration of storage and smoking history. The Netherlands – single hospital. October 1997 to May 1999. Pregnant women. 10-11 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 8 cases. Reference standard: CVS.
Index and comparator tests	Urinary hyperglycosylated hCG, (procedures previously described in Cole 1999a).
Follow-up	100% karyotyping.

Urine tests for Down's syndrome screening (Review)

Weinans 2000 (Continued)

Aim of study To investigate the value of H-hCG measurements in very early pregnancy (prior to 12 weeks' 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 stan- dard? All tests	Yes	CVS.
Partial verification avoid- ed? 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	Yes	Reference standard interpreted without knowledge of index test results.
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results.
Relevant clinical informa- tion? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results re- ported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

AFP: alpha-fetoprotein

βhCG: beta human chorionic gonadotrophin

CVS: chorionic villus sampling

hCG: human chorionic gonadotrophin

ITA: invasive trophoblast antigen

NT: nuchal translucency

PAPP-A: Pregnancy-associated plasma protein A

SD: standard deviation

uE3: unconjugated oestriol

Characteristics of excluded studies [ordered by study ID]

Study	Reason for exclusion
Abbas 1995	Unable to extract useful data.
Abdul-Hamid 2004	No Down's syndrome pregnancies.
Abraha 1999	Unable to extract useful data.
Adekunle 1999	Unable to extract useful information.
Aitken 1993	Unable to extract useful data.
Aitken 1996a	Fewer than 80% of pregnancies had gestational age confirmed by USS.
Aitken 1996b	Fewer than 80% of pregnancies had gestational age confirmed by USS.
Akbas 2001	Less than 5 Down's syndrome pregnancies.
Antona 1998	Likely fewer than 80% of pregnancies dated by USS.
Antsaklis 1999	Women screened at greater than 24 weeks' gestation.
Ashwood 1987	Unable to extract useful data.
Asrani 2005	Review article.
Audibert 2001b	Data were not relevant to this review - this study was not looking at urine tests for Down's syndrome screening
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.
Bar-Hava 2001	No Down's pregnancies in study population.
Barkai 1996	No Down's pregnancies in study population.
Barnabei 1995	No Down's pregnancies in study population.
Bartels 1988	Unable to extract useful data.
Bartels 1993	No Down's pregnancies in study population.
Barth 1991	Second trimester ultrasound study.

Study	Reason for exclusion
Baviera 2004	Unclear method of confirmation of gestational age.
Bazzett 1998	Male versus female fetuses.
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 2003a	Less than 80% of pregnancies dated by USS.
Benn 2003b	Editorial.
Benn 2005a	No Down's pregnancies included.
Benn 2005b	Mathematical model.
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.
Biggio 2004	Cost-effectiveness analysis.
Bindra 2002	Review article.
Blundell 1999	Unable to extract useful data.
Boots 1989	Population risk factor calculations.
Borruto 2002	Unable to extract useful data.
Boue 1990	Review article.
Bradley 1994	Screen-negative population gestations not confirmed by ultrasound.

Study	Reason for exclusion
Braithwaite 1996	Review article.
Brambati 1995	USS screening inclusive of women greater than 14 weeks' gestation.
Brambati 1996	Review article.
Brizot 1995a	Unable to extract useful data.
Brizot 1995b	Unable to extract useful data.
Brizzi 1989b	Second trimester ultrasound.
Brock 1990	Unable to extract useful data.
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.
Cans 1998	Second trimester ultrasound.
Carreras 1991	Second trimester ultrasound.
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.
Cheng 1993	Likely that fewer than 80% of gestational age confirmed by USS.
Cheng 1999	Case series. No Down's syndrome pregnancies in study population.
Cheng 2004a	No Down's syndrome pregnancies in study population.
Cheng 2004b	No Down's syndrome pregnancies in study population.
Chitayat 2002	Less than 5 Down's cases in study population.
Christiansen 2002	Unable to extract useful data.
Christiansen 2007	Unable to extract useful data.
Chung 2000	Less than 5 Down's syndrome pregnancies in study population.
CNGOF 1996	Unable to obtain translation.
Cole 1996	Review article.
Comas 2001	USS at greater than 14 weeks.

Study	Reason for exclusion
Comas 2002a	USS at greater than 14 weeks.
Comas 2002b	USS at greater than 14 weeks.
Comstock 2006	Unable to extract useful data.
Conde-Agudelo 1998	Review article.
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 1987a	Gestational age not confirmed by USS.
Cuckle 1987b	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.
D'Ottavio 1997	Second trimester USS.
Dancoine 2001	No Down's syndrome pregnancies in study population.
De Biasio 2000	Unable to extract useful information.
De Biasio, 1999	Unable to ascertain whether overlapping populations between several papers - attempted to contact author with no response.
De Biasio, 2001	Unable to ascertain whether overlapping populations between several papers - attempted to contact author with no response.
De Graaf 1991	Unable to extract useful data.
De Graaf 1999	Modelled data.
DeVore 2001	Second trimester ultrasound.
Dickerson 1994	Comment.
Dimaio 1987	Gestational age by USS only in screen-positive population.

Study	Reason for exclusion
Doran 1986	Ultrasound confirmation of gestational age performed in screen-positive women only.
Drugan 1996a	Second trimester ultrasound.
Drugan 1996b	Unable to extract useful data.
Drysdale 2002	Fewer than 5 Down's syndrome pregnancies in population.
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.
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.
Ghidini 1998	Comparison of male versus female fetuses.
Goldie 1995	Fewer than 80% of study population had gestational age confirmed by USS.
Gonçalves 2004	Greater than 14 weeks USS screening.
Goodburn 1994	Likely that fewer than 80% of pregnancies had gestational age estimated by USS.
Grozdea 2002	Unable to extract useful data.
Gyselaers 2004a	Less than 80% follow-up.
Gyselaers 2004b	Less than 80% follow-up.
Gyselaers 2006a	Unaffected pregnancies only.
Gyselaers 2006b	Unable to extract useful data.
Hackshaw 1995	No Down's syndrome pregnancies in population.
Hackshaw 2001	No Down's syndrome pregnancies in population
Haddow 1992	Less than 80% of pregnancies had gestational age confirmed by ultrasound scan.
Hafner 1995	Less than 5 Down's pregnancies in study population.
Hallahan 1998	Gestational age greater than 24 weeks.
Harrison 2006	Less than 80% of pregnancies had gestational age confirmed by USS.

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.
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.
Hogdall 1992	Unclear method of determination of gestational age. Unable to extract useful data.
Hong Kong Practitioner 2001	CME.
Howe 2000	Second trimester USS.
Hsiao 1991	Unable to obtain translation.
Hsieh 1999	No Down's syndrome pregnancies in study population.
Hsu 1997b	Adjustment factors.
Hsu 1998a	No Down's syndrome pregnancies in study population.
Hsu 1999b	No Down's pregnancies.
Huang 2003	No Down's syndrome pregnancies in study population.
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.
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.

Study	Reason for exclusion
Iles 1996	Review.
Ind 1994	Unable to extract useful data.
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.
Josefsson 1998	No Down's syndrome pregnancies in study population.
Jou 2001	Less than 5 Down's syndrome pregnancies in study population.
Kagan 2006	Screen-positive pregnancies only.
Kautzmann 1995	Fewer than 80% pregnancies had gestational age estimated by USS.
Keith 1992	Summary article.
Kelekci 2004	Less than 5 Down's syndrome pregnancies in population.
Kellner 1995a	Less than 5 Down's syndrome pregnancies in population.
Kellner 1995b	Less than 80% follow-up. Unable to ascertain proportion of population with gestational age confirmed by USS.
Kellner 1997	Assumption of normal karyotype without reference standard in significant proportion of control pregnancies.
Knight 1990	Review article.
Knight 2001	Validation of a specific assay.
Knight 2005	Less than 80% of pregnancies had gestational age confirmed by USS.
Koos 2006	Review article.
Kornman 1996	Less than 5 Down's syndrome pregnancies in population.
Kornman 1997	Unable to extract useful information.
Kramer 1998	No Down's syndrome pregnancies in study population.
Krantz 1996	Modelled data.
Krantz 2005	Adjustment factor.
Kulch 1993	No Down's cases in population.
Lai 1998	Modelled population.
Lai 2003	No Down's syndrome pregnancies in study population.

Study	Reason for exclusion
Laigaard 2006a	Unable to extract useful data.
Laigaard 2006b	Simulation.
Lam 1997	Unable to extract useful data.
Lam 1998	Fewer than 80% pregnancies had gestational age estimated by USS.
Lam 1999a	No Down's syndrome pregnancies in population.
Lam 1999b	Unable to extract useful data.
Lam 2000	Study of women's decisions about screening.
Lam 2001	Male versus female fetuses.
Lambert-Messerlian 1996	Fewer than 80% of pregnancies USS dated.
Lambert-Messerlian 1998	Unable to extract useful 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.
Liao 1997	Unable to obtain translation.
Liao 2001	Unable to extract useful data.
Lim 2002	Second trimester ultrasound.
Lippman 1987	Editorial.
Liu 2003	Unable to obtain translation.
Lustig 1988	Gestational age by LMP only.
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.
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
Mangione 2001	Abnormal screening results only.
Maymon 2001a	No Down's syndrome pregnancies in study population.
Maymon 2001b	No normal test results included therefore unable to extract meaningful data.
Maymon 2002	No Down's syndrome pregnancies in study population.
Maymon 2004	No Down's syndrome pregnancies in study population.
Maymon 2005	Modelled data.
McDuffie 1996	USS dating on screen-positive women only.
Meier 2002	Observed versus expected cases of Down's syndrome in a population.
Merkatz 1984	Gestational age not confirmed by USS.
Merz 2005	Editorial.
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.
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.
Muller 1994	No Down's syndrome pregnancies in study population.
Muller 1996b	Unable to extract useful data.
Muller 1999	Unable to extract useful data.
Muller 2002a	Getstional age greater than 24 weeks.
Muller 2002b	Unable to extract meaningful data - unable to separate double and triple test data.
Muller 2003	No Down's syndrome pregnancies in study population.

Study	Reason for exclusion
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.
Neveux 1996a	No Down's syndrome pregnancies in population.
Neveux 1996b	Unable to extract useful data.
Ng 2004	Unable to extract useful data.
Nicolaidis 1992	Study of outcomes of abnormal NT results.
Nicolaidis 2000	Review article.
Nicolaidis 2004	Review article.
Nicolaidis 2005a	Unable to obtain translation - appears to be a review article.
Nicolaidis 2005b	Unable to obtain translation - appears to be a review article.
Nicolaidis 2005c	Unable to obtain translation - appears to be a review article.
Nicolaidis 2005d	Unable to obtain translation - appears to be a review article.
Nicolaidis 2005e	Unable to obtain translation - appears to be a review article.
Nicolaidis 2005f	Review article.
Niemimaa 2001	No Down's pregnancies in study population.
Niemimaa 2002	No Down's syndrome pregnancies in population.
Niemimaa 2003	No Down's syndrome pregnancies in population.
Noble 1997	Unable to extract useful data.
Norgaard-Pedersen 1990	Less than 80% of gestational ages confirmed by USS.
Norton 1992	Unable to extract useful data.
O'Brien 1997a	No Down's syndrome pregnancies in population.
O'Brien 1997b	No Down's syndrome pregnancies in population.
Odibo 2004	Gestational age greater than 14 weeks in USS population.
Ognibene 1999	Unable to extract useful data.
Olajide 1989	Unable to extract useful data.
Onda 1996	Unable to extract useful data.

Study	Reason for exclusion
Onda 1998	Unable to extract useful data.
Onda 2000	Less than 80% follow-up.
Orlandi 2002	No Down's syndrome pregnancies in study population.
Palka 1998	Twin data used in calculation of the median.
Palomaki 1989	Fewer than 80% USS dated.
Palomaki 1993	No Down's syndrome pregnancies in population.
Palomaki 1994	No Down's syndrome pregnancies in population.
Palomaki 1996	Meta-analysis.
Palomaki 2005	Unable to extract meaningful data.
Panburana 2001	Less than 5 Down's syndrome pregnancies in population.
Pandya 1994	Study of outcomes of abnormal NT results.
Pandya 1995	Review article.
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.
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.
Pinette 2003	Women screened prior to recruitment.
Platt 2004	Unable to extract useful data.
Podobnik 1995	Abnormal results only.
Prefumo 2002	Comparison of prevalence and prediction.
Prefumo 2004	Comparison of a marker in women of different ethnic origins.
Price 1998	Unable to extract useful data.
Páez 2004	Unable to obtain translation.

Study	Reason for exclusion
Raty 2000	No Down's syndrome pregnancies in population.
Rembouskos 2004	Unable to extract useful data.
Ren 1992	Review article.
Renier 1998	Method of ascertainment of gestational age unclear. Twin gestations included in general population.
Resta 1990	Second trimester USS.
Reynders 1997	Fewer than 5 Down's cases.
Reynolds 1989	Explanation of mathematical techniques.
Reynolds 1999	Unable to extract useful data.
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 trial 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.
Saller 1997	Down's syndrome secondary to Robertsonian translocation only. No controls.

Study	Reason for exclusion
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.
Schlott 2006	Unable to extract useful data.
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.
Simon-Bouy 1999	Review article.
Simpson 1986	Gestational age confirmed by USS in less than 80% of population.
Smith 1990	Analysis of screen-positive results.
Smith 1996	Review/meta-analysis.
Smith 1999	Unable to extract useful data.
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.
Spencer 1985	Fewer than 80% USS dated.
Spencer 1991a	Likely fewer than 80% USS dated.
Spencer 1991b	Unable to extract useful data.

Study	Reason for exclusion
Spencer 1992	Unable to extract useful data.
Spencer 1993a	Fewer than 80% USS dated.
Spencer 1993b	No Down's pregnancies in study population.
Spencer 1993c	Unable to extract useful data.
Spencer 1993d	Fewer than 80% of pregnancies had gestational age confirmed by USS.
Spencer 1993e	Unable to extract useful data.
Spencer 1995	No Down's pregnancies in population.
Spencer 1996a	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.
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	Comparison of fetal sex.
Spencer 2001	No Down's syndrome pregnancies in population.
Spencer 2001a	Unable to extract useful data.
Spencer 2001b	Unable to extract useful data.
Spencer 2001c	Unable to extract useful data.
Spencer 2001d	No Down's syndrome pregnancies in population.
Spencer 2002a	No Down's pregnancies.

Study	Reason for exclusion
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.
Spong 1999	Comparison of male and female fetuses.
Stevens 1998	Literature review.
Stoll 1992	Review article.
Su 2002a	Unable to extract useful data.
Suchet 1995	Review article.
Suchy 1990	Unable to ascertain method of confirmation of gestational age.
Summers 2003a	Fewer than 80% had gestational age estimated by USS.
Summers 2003b	No Down's syndrome pregnancies in study population.
Suntharasaj 2005	Examination of inter-observer variation in NT scanning.
Sutton 2004	Unable to extract useful data.
Suzuki 1998	Unable to extract useful data.
Tabor 1987	Geststional age not confirmed by USS.
Tanski 1999	Information on screen-positive pregnancies only.
Thilaganathan 1998	No Down's syndrome pregnancies in study population.
Thilaganathan 1999	Editorial.
Tislaric 2002	No Down's syndrome pregnancies in population.
Torok 1997	Unable to extract useful data.

Study	Reason for exclusion
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 Heesch, 2006	No Down's syndrome pregnancies in study population. Software comparison study.
Van Lith 1991	Unable to extract useful data.
Van Lith 1993	Unable to extract useful data.
Van Lith 1994	Unable to extract useful data.
Veress 1986	Unable to extract useful data.
Veress 1988	Unable to extract useful data.
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	Fewer than 80% had gestational age estimated by USS
Wald 1994a	No Down's syndrome pregnancies in population.
Wald 1994b	Review article.
Wald 1996a	No Down's pregnancies.
Wald 1996b	Fewer than 80% had gestational age estimated by USS
Wald 1996d	No Down's syndrome pregnancies in population.
Wald 1996e	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.

Study	Reason for exclusion
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.
Wallace 1997	No Down's syndrome pregnancies in study population.
Ward 2005	Review article.
Watt 1996a	No Down's syndrome pregnancies in study population.
Watt 1996b	No Down's syndrome pregnancies in study population.
Weinans 2001	Unable to extract useful data.
Weinans 2004	Study of women's views on screening.
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.
Whitlow 1998a	Unable to extract useful data.
Whitlow 1998b	Unable to extract useful data.
Whitlow 1999	Unable to extract useful data.
Williamson 1994	Fewer than 80% had gestational age estimated by USS.
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.
Yagel 1998	Second trimester USS.
Yamamoto 2001a	Unable to extract useful data.
Yamamoto 2001b	Method of determination of gestational age unclear.
Yamamoto 2001c	Unable to extract useful data.
Yaron 2001	Male versus female fetuses.

Study	Reason for exclusion
Ye 1995	Unable to obtain translation.
Yoshida 2000	Fewer than 80% pregnancies had gestational age estimated by USS.
Zeitune 1991	Only aneuploid pregnancies included in study.
Zelop 2005	No Down's cases in population.
Zhao 1998	Unable to obtain translation.
Zoppi 2003	Inappropriate study design.

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 Betacore, 1st trimester urine test, 5% FPR	1	516
2 Betacore, 2nd trimester urine test, 5% FPR	6	9613
3 Betacore, 2nd trimester urine test, cutpoint mixed	7	10124
4 Gonadotropin, 2nd trimester urine test, risk 1:100	1	105
5 Gonadotropin, 2nd trimester urine test, risk 1:384	1	105
6 Gonadotropin, 2nd trimester urine test, 95% percentile	1	105
7 ITA, 1st trimester urine test, 5% FPR	2	579
8 ITA, 2nd trimester urine test, 3.74MoM	1	2051
9 ITA, 2nd trimester urine test, 5% FPR	3	2748
10 Total hCG, 1st trimester urine test, 5% FPR	1	516
11 Total hCG, 2nd trimester urine test, 5% FPR	1	390
12 Free βhCG, 1st trimester urine test, 5% FPR	1	516
13 Free βhCG, 2nd trimester urine test, 5% FPR	3	1517
14 Oestriol, 2nd trimester urine test, 5% FPR	2	1472
15 Betacore to oestriol ratio, 2nd trimester urine test, 5% FPR	2	1649
16 Betacore and oestriol, 2nd trimester 5% FPR	1	315

Test	No. of studies	No. of participants
17 AFP and ITA, 2nd trimester urine test, 3% FPR	1	524
18 AFP and ITA, 2nd trimester urine test, 5% FPR	1	524
19 AFP and ITA, 2nd trimester urine test, 10% FPR	1	524
20 AFP and ITA, 2nd trimester urine test, 15% FPR	1	524
21 AFP, uE3 and ITA, 2nd trimester urine test, 3% FPR	1	524
22 AFP, uE3 and ITA, 2nd trimester urine test, 5% FPR	1	524
23 AFP, uE3 and ITA, 2nd trimester urine test, 10% FPR	1	524
24 AFP, uE3 and ITA, 2nd trimester urine test, 15% FPR	1	524
25 Age, betacore, 2nd trimester urine test, 1% FPR	2	2083
26 Age, betacore, 2nd trimester urine test, 3% FPR	2	2083
27 Age, betacore, 2nd trimester urine test, 5% FPR	5	3419
28 Age, betacore, 2nd trimester urine test, 10% FPR	1	926
29 Age, betacore, 2nd trimester urine test, 15% FPR	1	953
30 Age, betacore, 2nd trimester urine test, 20% FPR	1	926
31 Age, ITA, 2nd trimester urine test, 5% FPR	1	1016
32 Age, oestriol, 2nd trimester urine test, 5% FPR	1	474
33 Age, free β hCG, 2nd trimester urine test, 5% FPR	2	879
34 Age, betacore to oestriol ratio, 2nd trimester urine test, 1% FPR	1	1157
35 Age, betacore to oestriol ratio, 2nd trimester urine test, 3% FPR	1	1157
36 Age, betacore to oestriol ratio, 2nd trimester urine test, 5% FPR	3	2088
37 Age, free β hCG to oestriol ratio, 2nd trimester urine test, 5% FPR	1	474
38 Age, oestriol and free β hCG, 2nd trimester, 5% FPR	1	474
39 Age, betacore to free β hCG ratio, 2nd trimester, 5% FPR	1	474
40 Age, betacore and oestriol, 2nd trimester 1% FPR	1	1157
41 Age, betacore and oestriol, 2nd trimester, 3% FPR	1	1157
42 Age, betacore and oestriol, 2nd trimester, 5% FPR	2	1631
43 Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:10	1	356
44 Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:20	1	356

Test	No. of studies	No. of participants
45 Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:30	1	356
46 Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:58	1	356
47 Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:270	1	356
48 Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:526	1	356

Test 1. Betacore, 1st trimester urine test, 5% FPR.

Test 2. Betacore, 2nd trimester urine test, 5% FPR.

Test 3. Betacore, 2nd trimester urine test, cutpoint mixed.

Test 4. Gonadotropin, 2nd trimester urine test, risk 1:100.

Review: Urine tests for Down's syndrome screening
Test: 4 Gonadotropin, 2nd trimester urine test, risk 1:100

Test 5. Gonadotropin, 2nd trimester urine test, risk 1:384.

Review: Urine tests for Down's syndrome screening
Test: 5 Gonadotropin, 2nd trimester urine test, risk 1:384

Test 6. Gonadotropin, 2nd trimester urine test, 95% percentile.

Review: Urine tests for Down's syndrome screening
Test: 6 Gonadotropin, 2nd trimester urine test, 95% percentile

Test 7. ITA, 1st trimester urine test, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 7 ITA, 1st trimester urine test, 5% FPR

Test 8. ITA, 2nd trimester urine test, 3.74MoM.

Review: Urine tests for Down's syndrome screening
Test: 8 ITA, 2nd trimester urine test, 3.74MoM

Test 9. ITA, 2nd trimester urine test, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 9 ITA, 2nd trimester urine test, 5% FPR

Test 10. Total hCG, 1st trimester urine test, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 10 Total hCG, 1st trimester urine test, 5% FPR

Test 11. Total hCG, 2nd trimester urine test, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 11 Total hCG, 2nd trimester urine test, 5% FPR

Test 12. Free β hCG, 1st trimester urine test, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 12 Free β hCG, 1st trimester urine test, 5% FPR

Test 13. Free β hCG, 2nd trimester urine test, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 13 Free β hCG, 2nd trimester urine test, 5% FPR

Test 14. Oestriol, 2nd trimester urine test, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 14 Oestriol, 2nd trimester urine test, 5% FPR

Test 15. Betacore to oestriol ratio, 2nd trimester urine test, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 15 Betacore to oestriol ratio, 2nd trimester urine test, 5% FPR

Test 16. Betacore and oestriol, 2nd trimester 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 16 Betacore and oestriol, 2nd trimester 5% FPR

Test 17. AFP and ITA, 2nd trimester urine test, 3% FPR.

Review: Urine tests for Down's syndrome screening
Test: 17 AFP and ITA, 2nd trimester urine test, 3% FPR

Test 18. AFP and ITA, 2nd trimester urine test, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 18 AFP and ITA, 2nd trimester urine test, 5% FPR

Test 19. AFP and ITA, 2nd trimester urine test, 10% FPR.

Review: Urine tests for Down's syndrome screening
Test: 19 AFP and ITA, 2nd trimester urine test, 10% FPR

Test 20. AFP and ITA, 2nd trimester urine test, 15% FPR.

Review: Urine tests for Down's syndrome screening
Test: 20 AFP and ITA, 2nd trimester urine test, 15% FPR

Test 21. AFP, uE3 and ITA, 2nd trimester urine test, 3% FPR.

Review: Urine tests for Down's syndrome screening
Test: 21 AFP, uE3 and ITA, 2nd trimester urine test, 3% FPR

Test 22. AFP, uE3 and ITA, 2nd trimester urine test, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 22 AFP, uE3 and ITA, 2nd trimester urine test, 5% FPR

Test 23. AFP, uE3 and ITA, 2nd trimester urine test, 10% FPR.

Review: Urine tests for Down's syndrome screening
Test: 23 AFP, uE3 and ITA, 2nd trimester urine test, 10% FPR

Test 24. AFP, uE3 and ITA, 2nd trimester urine test, 15% FPR.

Review: Urine tests for Down's syndrome screening
Test: 24 AFP, uE3 and ITA, 2nd trimester urine test, 15% FPR

Test 25. Age, betacore, 2nd trimester urine test, 1% FPR.

Review: Urine tests for Down's syndrome screening
Test: 25 Age, betacore, 2nd trimester urine test, 1% FPR

Test 26. Age, betacore, 2nd trimester urine test, 3% FPR.

Review: Urine tests for Down's syndrome screening
Test: 26 Age, betacore, 2nd trimester urine test, 3% FPR

Test 27. Age, betacore, 2nd trimester urine test, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 27 Age, betacore, 2nd trimester urine test, 5% FPR

Test 28. Age, betacore, 2nd trimester urine test, 10% FPR.

Review: Urine tests for Down's syndrome screening
Test: 28 Age, betacore, 2nd trimester urine test, 10% FPR

Test 29. Age, betacore, 2nd trimester urine test, 15% FPR.

Review: Urine tests for Down's syndrome screening
Test: 29 Age, betacore, 2nd trimester urine test, 15% FPR

Test 30. Age, betacore, 2nd trimester urine test, 20% FPR.

Review: Urine tests for Down's syndrome screening
Test: 30 Age, betacore, 2nd trimester urine test, 20% FPR

Test 31. Age, ITA, 2nd trimester urine test, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 31 Age, ITA, 2nd trimester urine test, 5% FPR

Test 32. Age, oestriol, 2nd trimester urine test, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 32 Age, oestriol, 2nd trimester urine test, 5% FPR

Test 33. Age, free β hCG, 2nd trimester urine test, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 33 Age, free β hCG, 2nd trimester urine test, 5% FPR

Test 34. Age, betacore to oestriol ratio, 2nd trimester urine test, 1% FPR.

Review: Urine tests for Down's syndrome screening
Test: 34 Age, betacore to oestriol ratio, 2nd trimester urine test, 1% FPR

Test 35. Age, betacore to oestriol ratio, 2nd trimester urine test, 3% FPR.

Review: Urine tests for Down's syndrome screening
Test: 35 Age, betacore to oestriol ratio, 2nd trimester urine test, 3% FPR

Test 36. Age, betacore to oestriol ratio, 2nd trimester urine test, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 36 Age, betacore to oestriol ratio, 2nd trimester urine test, 5% FPR

Test 37. Age, free β hCG to oestriol ratio, 2nd trimester urine test, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 37 Age, free β hCG to oestriol ratio, 2nd trimester urine test, 5% FPR

Test 38. Age, oestriol and free β hCG, 2nd trimester, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 38 Age, oestriol and free β hCG, 2nd trimester, 5% FPR

Test 39. Age, betacore to free β hCG ratio, 2nd trimester, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 39 Age, betacore to free β hCG ratio, 2nd trimester, 5% FPR

Test 40. Age, betacore and oestriol, 2nd trimester 1% FPR.

Review: Urine tests for Down's syndrome screening
Test: 40 Age, betacore and oestriol, 2nd trimester 1% FPR

Test 41. Age, betacore and oestriol, 2nd trimester, 3% FPR.

Review: Urine tests for Down's syndrome screening
Test: 41 Age, betacore and oestriol, 2nd trimester, 3% FPR

Test 42. Age, betacore and oestriol, 2nd trimester, 5% FPR.

Review: Urine tests for Down's syndrome screening
Test: 42 Age, betacore and oestriol, 2nd trimester, 5% FPR

Test 43. Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:10.

Review: Urine tests for Down's syndrome screening
Test: 43 Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:10

Test 44. Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:20.

Review: Urine tests for Down's syndrome screening
Test: 44 Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:20

Test 45. Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:30.

Review: Urine tests for Down's syndrome screening
Test: 45 Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:30

Test 46. Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:58.

Review: Urine tests for Down's syndrome screening
Test: 46 Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:58

Test 47. Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:270.

Review: Urine tests for Down's syndrome screening
Test: 47 Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:270

Test 48. Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:526.

Review: Urine tests for Down's syndrome screening
Test: 48 Age, AFP and betacore to oestriol ratio, 2nd trimester, risk 1:526

ADDITIONAL TABLES**Table 1. Direct comparisons of the diagnostic accuracy of five urine tests in combination with maternal age**

Ratio of DORs (95% CI); P values (studies)	Second trimester AFP and β -core fragment to oestriol ratio, risk 1:58	Second trimester β -core fragment and oestriol, 5% FPR	Second trimester ITA, 5% FPR	Second trimester β -core fragment to oestriol ratio, 5% FPR
Second trimester β -core fragment and oestriol, 5% FPR	–			
Second trimester ITA, 5% FPR	–	–		
Second trimester β -core fragment to oestriol ratio, 5% FPR	–	1.5 (0.7 to 3.0); P = 0.27 (K = 2)		
Second trimester β -core fragment, 5% FPR	–	2.2 (1.1 to 4.5); P = 0.02 (K = 2)	–	1.5 (0.8 to 2.8); P = 0.21 (K = 3)

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 column by the DOR for the test in the row. If the ratio of DORs is greater than one, then the diagnostic accuracy of the test in the column is higher than that of the test in the row; if the ratio is less than one, the diagnostic accuracy of the test in the row is higher than that of the test in the column.

AFP: alpha-fetoprotein; **CI:** confidence interval; **DORs:** diagnostic odds ratio; **FPR:** false positive rate; **ITA:** invasive trophoblast antigen

Table 2. Indirect comparisons of the diagnostic accuracy of five urine tests in combination with maternal age

Ratio of DOR (95% CI); P value	Second trimester AFP and β -core fragment to oestriol ratio, risk 1:58	Second trimester β -core fragment and oestriol, 5% FPR	Second trimester ITA, 5% FPR	Second trimester β -core fragment to oestriol
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Table 2. Indirect comparisons of the diagnostic accuracy of five urine tests in combination with maternal
age (Continued)

			ratio, 5% FPR			
			Studies	1	2	3
			DOR (95% CI)	186 (22, 1560)	50 (30 to 84)	43 (17 to 110)
						38 (24 to 59)
Second trimester β-core fragment and oestriol, 5% FPR	2	50 (30 to 84)	3.7 (0.4 to 33.0); P = 0.24			
Second trimester ITA, 5% FPR	1	43 (17 to 110)	4.3 (0.4 to 44.0); P = 0.22	1.2 (0.4 to 3.4); P = 0.78		
Second trimester β-core fragment to oestriol ratio, 5% FPR	3	38 (24 to 59)	4.9 (0.6 to 43.4); P = 0.15	1.3 (0.7 to 2.6); P = 0.41	1.1 (0.4 to 3.2); P = 0.80	
Second trimester β-core fragment, 5% FPR	5	25 (18 to 36)	7.3 (0.8 to 63.1); P = 0.07	2.0 (1.1 to 3.7); P = 0.03	1.7 (0.6 to 4.6); P = 0.30	1.5 (0.8 to 2.6); P = 0.18

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

AFP: alpha-fetoprotein; **CI:** confidence interval; **DORs:** diagnostic odds ratio; **FPR:** false positive rate; **ITA:** invasive trophoblast antigen

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

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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 STANDARDS\$

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

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

YT undertook statistical analyses and wrote parts of the first draft of the review.

DECLARATIONS OF INTEREST

S Kate Alldred: none known

Zarko Alfirevic: none known

Jonathan J Deeks: none known

James P Neilson: none known

Boliang Guo: none known

Mary Pennant: none known

Susanna Wisniewski: none known

Yemisi Takwoingi: none known

SOURCES OF SUPPORT

Internal sources

- University of Birmingham, Other.

Funding of research time for BG, MP, SW, YT and JD

External sources

- NIHR Health Technology Assessment Programme, UK, Other.

Project grant

- NIHR Health Technology Assessment Programme, UK, Other.

Funding for the Cochrane Reviews of Diagnostic Test Accuracy Support Unit, based at the University of Birmingham (JD).

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. Where such information was found, it was difficult to extract meaningful data to allow for comparison between studies because 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 tests; 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, so that we focused on key tests and test combinations by a) only meta-analysing tests that were included in four or more studies, or b) showed more than 70% sensitivity with at least a 95% specificity. In addition, a requirement that a minimum of 10 studies for a single test was required before subgroup analysis was undertaken. Consequently several possible sources of heterogeneity were not investigated due to lack of data.

NOTES

This review belongs to a suite of planned systematic diagnostic test reviews examining antenatal screening for Down's syndrome which include four other titles: *First trimester serum tests for Down's syndrome screening*; *Second trimester serum tests for Down's syndrome screening* (Alldred 2012); *First trimester serum and ultrasound tests for Down's syndrome screening*; and *First and second trimester serum*

tests with and without first trimester ultrasound tests for Down's syndrome screening. The plans for these reviews were described in a generic protocol (Aldred 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)

Biomarkers [*urine]; Chorionic Gonadotropin [urine]; Down Syndrome [*diagnosis]; Estriol [urine]; False Positive Reactions; Gonadotropins [urine]; Maternal Age; Predictive Value of Tests; Pregnancy Trimester, First [*urine]; Pregnancy Trimester, Second [*urine]; Sensitivity and Specificity; alpha-Fetoproteins [urine]

MeSH check words

Female; Humans; Pregnancy