ANALYSIS OF CELL-FREE DNA IN MATERNAL BLOOD IN SCREENING FOR ANEUPLOIDIES: UPDATED META-ANALYSIS

Short title: Cell-free DNA in screening for aneuploidies

Key words: Cell-free fetal DNA; Non-invasive prenatal testing; Trisomy 21; Trisomy 18; Trisomy 13; Turner syndrome; Fetal aneuploidy.

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ABSTRACT

Objective: To review clinical validation or implementation studies of maternal blood cell-free (cf) DNA analysis and define the performance of screening for fetal trisomies 21, 18 and 13 and sex chromosome aneuploidies.

Data sources: Searches of PubMed, Embase and the Cochrane library were performed to identify all peer-reviewed articles on cfDNA testing in screening for aneuploidies between January 2011, when the first such study was published and 31 December 2016.

Results: In total, 35 relevant studies were identified and these reported cfDNA results in relation to fetal karyotype from invasive testing or clinical outcome. In the combined total of 1,963 cases of trisomy 21 and 225,032 non-trisomy 21 singleton pregnancies the pooled weighted detection rate (DR) and false positive rate (FPR) were 99.7% (95% CI 99.1-99.9%) and 0.04% (95% CI 0.02-0.08%), respectively. In a total of 560 cases of trisomy 18 and 212,019 unaffected singleton pregnancies the pooled weighted DR and FPR were 98.2% (95% CI 95.5-99.2%) and 0.05% (95% CI 0.03-0.07%). In a total of 119 cases of trisomy 13 and 212,883 unaffected singleton pregnancies the pooled weighted DR and FPR were 99.0% (95% CI 65.8-100%) and 0.04% (95% CI 0.02-0.07%). In a total of 36 cases of monosomy X and 7,677 unaffected singleton pregnancies the pooled weighted DR and FPR were 95.8% (95% CI 70.3-99.5%) and 0.14% (95% CI 0.05-0.38%). In a combined total of 17 cases of sex chromosome abnormalities other than monosomy X and 5,383 unaffected singleton pregnancies the pooled weighted DR and FPR were 100% (95% CI 83.6-100%) and 0.003% (95% CI 0-0.07%). For twin pregnancies, in a total of 24 cases of trisomy 21 and 1,111 unaffected cases the DR was 100% (95% 95.2-100%) and FPR was 0% (95% CI 0-0.003%).

Conclusion: Screening by analysis of cfDNA in maternal blood in singleton pregnancies could detect >99% of fetuses with trisomy 21, 98% of trisomy 18 and 99% of trisomy 13 at a combined FPR of 0.13%. The number of reported cases of sex chromosome abnormalities is too small for accurate assessment of performance of screening. In twin pregnancies performance of screening for trisomy 21 is encouraging but the number of cases reported is small.

Introduction

Several studies in the last five years have reported the clinical validation and/or implementation of analyzing cell-free (cf) DNA in maternal blood in screening for trisomies 21, 18 and 13 and sex chromosome aneuploidies. In previous meta-analyses[1,2], we reported the results from studies published between January 2011 and 4 January 2015.

The objective of this meta-analysis is to update the results with inclusion of studies that were published up to 31 December 2016. In this analysis we excluded case-control studies because they tend to introduce an optimistic bias to the estimates of diagnostic performance, but they were the first studies to be carried out after introduction of cfDNA testing and in this respect they could be underestimating the performance of an evolving technique. Pooled detection rate (DR) and false positive rate (FPR) were calculated using bivariate random-effects regression models, and heterogeneity was investigated.

Systematic review and meta-analysis

Literature search and study selection

Searches of Pubmed, Embase and The Cochrane Library were performed, with a limit to English language, to identify all peer-reviewed articles published on clinical validation or implementation of maternal cfDNA testing in screening for aneuploidies. The search period was between January 2011, when the first such study was published and 31 December 2016. The following search terms were used: “maternal blood cell free DNA”, “cell free DNA prenatal”, “noninvasive prenatal”, “non-invasive prenatal” or “non invasive prenatal” in combination with “diagnosis”, “testing” or “detection”.

The abstracts of citations were examined by two reviewers (M.M.G., V.A.) to identify all potentially relevant articles which were then examined in full text. Reference lists of relevant original and review articles were searched for additional reports. Agreement about potential relevance was reached by consensus and by consultation with the third reviewer (K.H.N.).

The inclusion criteria were peer-review studies reporting on clinical validation or implementation of maternal cfDNA testing in screening for aneuploidies, in which data on pregnancy outcome were provided for more than 85% of the study population to avoid reporting bias. Case-control studies, proof-of principle articles and studies in which the laboratory scientists carrying out the tests were aware of fetal karyotype or pregnancy outcome were excluded.

Data extraction and meta-analysis of data from all studies

Data regarding sample size, gestational age at analysis, method used for cfDNA testing and DR or sensitivity and FPR or specificity for non-mosaic trisomies 21,18, 13 and sex chromosome aneuploidies (SCA) was obtained from each study included in the systematic review and documented in contingency tables. In the construction of these tables we used the results from the cfDNA test and excluded those cases where the test failed to give a result. In the calculation of FPR we included all euploid and aneuploid cases other than the aneuploidy under investigation. Authors were contacted when clarifications were required in the interpretation of the data.

We extracted data from the primary studies to obtain the four cell values of a diagnostic 2×2 table to calculate test accuracy measures of DR and FPR. The analyses were stratified according to the different conditions (trisomy 21, trisomy 18, trisomy 13, monosomy X and sex chromosome aneuploidy other than monosomy X for singleton pregnancies and trisomy 21 for twin pregnancies). We calculated DR and FPR with corresponding 95% CIs for individual studies and displayed them in forest plots to investigate heterogeneity. We pooled the DR and FPR estimates using bivariate random-effects regression models. The bivariate model assumes that logit transformations of DR and FPR are negatively correlated and follow a bivariate normal distribution[4]. We computed the positive and negative likelihood ratios from the pooled estimates of DR and FPR. Where there was insufficient number of studies to reliably estimate all the parameters in the bivariate model we computed average DR and FPR values by using a univariate random-effects model.

Heterogeneity among studies was quantified with the variance of the logit of accuracy indices as estimated by the bivariate model. When a univariate model was used, we assessed heterogeneity between study results using the I^2 statistic as previously described[1]. We selected the following factors as potential sources of a priori heterogeneity: methods for analysis of cfDNA in maternal blood [massively parallel shotgun sequencing (MPSS), chromosome-selective sequence analysis (CSS) and single nucleotide polymorphism only based analysis (SNP), population risk (high-risk, routine, mixed-risk) and gestational age of assessment (first-trimester vs all trimesters)]. We performed a subgroup analysis in the case of trisomies 21 and 18 by method used for cfDNA testing (MPSS, CSS or SNP), gestational age at testing (first-trimester vs any gestational age) and type of population examined (high-risk, routine population or mixed). Summary DR and FPR estimates for each subgroup were generated, along with their 95% CIs. We investigated heterogeneity by adding covariate terms to the bivariate model to assess the effect of a covariate on accuracy.

We assessed publication bias by representing diagnostic odds ratio (a single measure of diagnostic accuracy) against the effective sample size. With no bias the plot should show an inverted symmetrical funnel shape (a vertical pattern would indicate no publication bias whereas slope would indicate the degree of bias). The degree of asymmetry was statistically assessed by regression of the logarithm of diagnostic odds ratio on the inverse of the square root of the effective sample size, weighted by this sample size. A p value <0.10 indicates significant publication bias[5].

We conducted statistical analyses using the Metandi and Midas programs for the STATA software (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP) and the open source Mada written in R (R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

Results

Data sources

The search identified 7,759 potentially relevant citations (Figure 1). The following groups were excluded: first, conference abstracts rather than peer-reviewed papers (n=1,070), second, non-relevant publications (n=2,001), third, review articles or opinions (n=154), fourth, proof-of-principle studies reporting laboratory techniques rather than clinical validation of a predefined method of maternal blood cfDNA analysis (n=29) (Table S1), fifth, studies on clinical implementation of cfDNA testing in screening for

aneuploidies in which pregnancy outcome data were provided for less than 85% of the study population (n=29) (Table S2) and six, case-control studies (n=35) (Table S3).

In total, 35 relevant studies were identified and these were used for the meta-analysis on the performance of cfDNA testing in screening for aneuploidies[6-40]. These studies reported cfDNA results in relation to fetal karyotype from invasive testing or clinical outcome.

In two of the 35 studies some of the maternal blood samples for the cfDNA analysis were obtained after the invasive test[16,19]. In 28 studies it was either explicitly stated[7-14,15,18,20-22,27,32,33,36,38] or assumed on the basis of the described methodology[17,26,28-31,34,35,37,39] that if an invasive test was carried out the samples for cfDNA analysis were obtained before the invasive test. In five studies it is uncertain if invasive testing was before or after maternal blood sampling for the cfDNA test[6,23-25,40].

The characteristics of the included studies are summarized in Table 1.

Methodological quality of the selected studies

The methodological quality of the selected studies, assessed by QUADAS-2[41] is illustrated in Figure 2. This tool comprises four domains; each one is assessed in terms of risk of bias and the first three are also assessed in terms of concerns regarding applicability; the results of individual studies are given in Table 2.

In the assessment of risk of bias, the first domain relates to patient selection and a study was considered to be at low-risk of bias if the cfDNA test was carried out in a consecutive or random sample of patients; most studies were classified as being at high-risk of bias because either the samples were not explicitly stated to have been consecutive or selected at random. The second domain relates to the index test and a study was considered to be at low-risk of bias if the cfDNA test was carried out and the results given by the laboratory without prior knowledge of the fetal karyotype or pregnancy outcome; studies in which this was not explicitly stated in the paper were classified as being at high-risk of bias. The third domain relates to the reference standard and a study was considered to be at low-risk of bias if the method of diagnosing the chromosomal abnormality under investigation was able to give the correct answer; for trisomies 21, 18 or 13 we accepted this to be true if the diagnosis was based on prenatal or postnatal karyotyping in the case of affected fetuses and karyotyping or examination of the neonate in the case of unaffected fetuses. The risk of bias was also considered to be low for most studies on sex chromosome aneuploidies because the karyotype was ascertained from invasive testing; however, studies in which the assumption of normal karyotype was based on clinical examination at birth rather than karyotyping were classified as being at high-risk of bias because unlike the situation with trisomies 21, 18 and 13, most neonates with sex chromosome aneuploidies are often phenotypically normal. The fourth domain relates to flow and timing and a study was considered to be at low-risk of bias if firstly, in the calculation of performance of screening all patients in the study population had a result from both the cfDNA test and pregnancy outcome and secondly, if the method of classifying the outcome result (invasive testing or clinical examination) was the same in all cases in the study population. Most studies were classified as being at high-risk of bias because cfDNA testing was not carried out or did not provide results in all cases and/or there was no complete follow up and/or the method of determining outcome was not the same in all cases.

In the assessment of concerns regarding applicability of cfDNA testing to screening in the general population the first domain relates to patient selection and most studies were classified as being at high-risk of concerns regarding applicability because there was prior screening by another method; only a few studies where the test was carried out in the general population were classified as being at low-risk. In terms of the second domain on index test, all studies classified as being at low-risk of bias were also considered as being at low-risk of concerns regarding applicability; only a few studies were classified as high-risk. Similarly, for the third domain on reference standard, all studies reporting on trisomies 21, 18 or 13 were classified as being at low-risk of concerns regarding applicability; those reporting on sex chromosome aneuploidies without karyotyping of all cases in the study population were classified as high-risk of concerns regarding applicability.

Method of analyzing samples

The studies included in the meta-analysis used one of three methods for analysis of cfDNA in maternal blood: MPSS, CSS, SNP (Table 1). Other methods of examining fetoplacental nucleic acids in maternal blood have been investigated, but these have not yet been implemented in clinical practice.

Nature of the studies

Most of the studies included in this meta-analysis were prospective or retrospective cohort studies in high-risk pregnancies, nine studies reported on routine screening in the general population and five studies examined a mixture of high-risk and routine populations (Table 1).

No result rate from cfDNA testing

One issue with cfDNA testing as a method of screening for aneuploidies is failure to provide a result. There are essentially three reasons for such failure. Firstly, problems with blood collection and transportation of the samples to the laboratory, including inadequate blood volume, hemolysis, incorrect labelling of tubes and delay in arrival to the laboratory. Secondly, low fetal fraction, usually below 4%, and thirdly, assay failure for a variety of reasons, including failed DNA extraction, amplification and sequencing.

Data on the no result rate from the studies included in the meta-analysis are summarized in Table 3. Data relating to blood collection and transportation of the samples were provided by nine studies and the reported rates ranged from 0.03% to 11.1%. Data on failure to provide results for samples that were analyzed were provided by 31 of the studies and the reported rates ranged from 0% to 12.2%. In 11 of the 35 studies further details were given for the reason of the failure being low fetal fraction and the reported rates ranged from 0.1% to 6.1%.

On the basis of the published data, it is not possible to offer an explanation for the wide range in failure rates between studies or to draw conclusions on the possible relationship between the no result rate and the method used for the analysis of samples (Figure 4) or gestational age at sampling. However, the data showed by the three studies that reported the no result rate separately for trisomies and sex chromosome aneuploidies[6,20,28] suggest that the rate for the latter is higher; the rate was 5.9% (418 of 7,097) for trisomies and 11.7% (559 of 4,780) for sex chromosome aneuploidies ($P<0.0001$).

Meta-analysis and performance of screening for aneuploidies

The DR and FPR for each study, weighted pooled data and heterogeneity between studies (variance of the logit sensitivity and specificity, or I^2 statistic when univariate analysis was used) are provided in Tables 4-9 and illustrated in Figures 5-10. The publication bias of the studies is also given in Tables 4-9 and assessed graphically using funnel plots in Figure 3. There was a high likelihood of publication bias for all conditions (p value < 0.10).

Trisomy 21

A total of 30 studies reported on the performance of screening by cfDNA analysis for trisomy 21 in a combined total of 1,963 cases of trisomy 21 and 225,032 non-trisomy 21 singleton pregnancies (Table 4). In individual studies the DR varied between 94.4% and 100% and the FPR varied between 0% and 0.94%. The pooled weighted DR and FPR were 99.7% (95% CI 99.1-99.9%) and 0.04% (95% CI 0.02-0.08%), respectively.

Trisomy 18

A total of 25 studies reported on the performance of screening by cfDNA analysis for trisomy 18 in a combined total of 560 cases of trisomy 18 and 212,019 non-trisomy 18 singleton pregnancies (Table 5). In individual studies the DR varied between 88.0% and 100% and the FPR varied between 0% and 0.22%. The pooled weighted DR and FPR were 98.2% (95% CI 95.5-99.2%) and 0.05% (95% CI 0.03-0.07%), respectively.

Trisomy 13

A total of 23 studies reported on the performance of screening by cfDNA analysis for trisomy 13 in a combined total of 119 cases of trisomy 13 and 212,883 non-trisomy 13 singleton pregnancies (Table 6). In individual studies the DR varied between 40.0% and 100% and the FPR varied between 0% and 0.25%. The pooled weighted DR and FPR were 99.0% (95% CI 65.8-100%) and 0.04% (95% CI 0.02-0.07%), respectively.

Monosomy X

A total of 11 studies reported on the detection of monosomy X by cfDNA analysis for a combined total of 36 cases of monosomy X and 7,677 with no monosomy X singleton pregnancies (Table 7). In individual studies the DR varied between 66.7% and 100% and the FPR varied between 0% and 0.49%. The pooled weighted DR and FPR were 95.8% (95% CI 70.3-99.5%) and 0.14% (95% CI 0.05-0.38%), respectively.

Sex chromosome aneuploidies other than monosomy X

A total of 8 studies reported on the performance of screening by cfDNA analysis for sex chromosome abnormalities other than monosomy X in a combined total of 17 affected and 5,383 non-sex chromosome aneuploidy singleton pregnancies (Table 8). The pooled weighted DR and FPR were 100% (95% CI 83.6-100%) and 0.003% (95% CI 0-0.07%), respectively.

Studies in twin pregnancies

Five studies reported on the performance of screening by cfDNA analysis for trisomies in twin pregnancies (Table 9). In a combined total of 24 cases of trisomy 21 and 1,111 euploid pregnancies the DR was 100% (95% 95.2-100%) and FPR was 0% (95% CI 0-0.003%). Additionally, there were 14 trisomy 18 pregnancies of which 13 were correctly

classified and also one case of trisomy 13 that was wrongly classified as non-trisomic [18,21,37].

Subgroup analysis on performance of screening for trisomies 21 and 18

In the case of trisomies 21 and 18 there were sufficient data to allow subgroup analyses (Table 10). There were no significant differences in performance of screening in relation to method used for cfDNA testing (MPSS, CSS or SNP), gestational age at testing (first-trimester vs any gestational age) and type of population examined (high-risk, routine population or mixed).

Discussion

Performance of screening

The finding of this meta-analysis demonstrate that screening for trisomies in singleton pregnancies by cfDNA in maternal blood could detect >99% of fetuses with trisomy 21, 98% of trisomy 18 and 99% of trisomy 13 at a combined FPR of 0.13%. The combined total number of trisomies 21 and 18 and unaffected pregnancies was large and the heterogeneity between studies was low. In the case of trisomy 13 the total number of affected cases was only 119 and in individual studies the DR varied between 40% and 100% and the FPR varied between 0% and 0.25%. The number of reported cases of sex chromosome abnormalities is too small for accurate assessment of performance of screening. In twin pregnancies performance of screening for trisomy 21 is encouraging but the number of cases reported is small.

Most of the studies included in this meta-analysis were in high-risk pregnancies and they were not confined to pregnancies in the first-trimester; however, subgroup analysis of cfDNA testing in singleton pregnancies for trisomies 21 and 18 demonstrated no significant difference in performance of screening between high-risk and routine or mixed populations and between those examined in the first-trimester and those examined at any stage in pregnancy. Similarly, there was no obvious difference in performance of screening between the MPSS, CSS or SNP methods for cfDNA testing.

In this review there are three improvements in comparison to our two previous ones.[1,2] First, many recent studies have been included. Second, we excluded case-control studies because of their trend to overestimation of test performance but equally important, because they were the first published studies and therefore the technologies used at that time were likely to have been worse than the current ones. Third, we used a bivariate, rather than univariate, model to analyse the data to take into account not only the between-study heterogeneity in DR and FPR but also the negative correlation between these two statistics.

Methodological quality of the selected studies

In the assessment of methodological quality by QUADAS-2[41], most studies were considered to be at high-risk of bias and at high-risk of concerns regarding applicability in relation to patient selection because they were performed in high-risk populations. However, the ability to detect aneuploidy with cfDNA analysis is dependent upon assay precision and fetal DNA percentage in the sample rather than the prevalence of the disease in the study population. This is supported by the finding that the performance of the test in the studies that were carried out in a general population was similar to that of studies in high-risk pregnancies. Most studies were also classified as being at high-risk

of bias in relation to flow and timing. This is essentially because cfDNA testing did not provide results in all cases, there was no complete follow up, or the method of determining outcome was not the same in all cases. However, such criticisms could be applied to any clinical study; all methods of traditional screening occasionally fail to give a result and no screening study in pregnancy can have complete follow up, especially because some women miscarry and karyotyping is not carried out. There is also an increased risk of bias in those studies where some of the samples for the cfDNA test were taken after chorionic villous sampling had been carried out;[16,19] the invasive procedure can cause a small but significant increase in the fetal fraction[42].

The real issue in relation to the failure rate in cfDNA testing is whether this is higher in aneuploid compared to euploid fetuses; studies examining this issue reported that the failure rate in trisomy 21 pregnancies is similar to that in euploid pregnancies, but in trisomies 18 and 13 the rate is increased[43] thereby introducing bias if only the cases with results are included in the calculation of the performance of screening. In the context of the method of determining outcome, most screening studies inevitably rely on karyotyping for diagnosis of trisomies 21, 18 and 13 and on clinical examination of the neonate for exclusion of these trisomies. The risk of bias in these cases is low because it is very unlikely that the diagnosis would be missed by clinical examination alone. In contrast, diagnosis or exclusion of sex chromosomal aneuploidies by clinical examination of the neonate is not reliable and consequently there are true concerns of high-risk of bias in relation to both the reference standard and flow and timing in the studies that did not rely entirely on karyotyping.

Failure rate

One issue with cfDNA testing as a method of screening for aneuploidies is failure to provide a result. However, the failure rate or the reasons for such failure were not consistently reported in the various studies included in this meta-analysis. The reported rates of inadequate blood collection and transportation of the samples ranged from 0.03% to 11.1% and the rates of failure to provide results for samples that were analyzed ranged from 0% to 12.2%. On the basis of the published data, it is not possible to offer an explanation for such wide range in failure rates between studies.

The main reason for failed result is low fetal fraction and the main determinants of low fetal fraction are maternal obesity and small placental mass. In trisomies 18 and 13, but not in trisomy 21, the fetal fraction is lower and the rate of failed cfDNA test is higher than in unaffected pregnancies.[43] Consequently, pregnancies with a failed test can be considered as being at increased risk for trisomies 18 and 13, but not for trisomy 21. The management of pregnancies with failed cfDNA test should essentially depend on the reason for carrying out such test in the first place. If there was prior screening with a low-risk result, the preferred option would be to repeat the cfDNA test and explain to the parents that such testing would provide a result in >60% of cases. Some patients would prefer to avoid any further testing because of the associated anxiety; in these patients and in those with a failed second cfDNA test it would be advisable to carry out a detailed ultrasound scan for features of trisomies 18 and 13 and in the presence of such features invasive testing should be considered. If prior screening had provided a high-risk result but there are no ultrasound features of an aneuploidy, most patients would prefer repeat cfDNA testing but a few would select to have invasive testing.[43]

Clinical implications

There is clear evidence that in singleton pregnancies the performance of screening for trisomies 21, 18 and 13 by cfDNA testing is superior, both in terms of higher DR and

substantially lower FPR, to that of all other methods combining maternal age, first- or second-trimester ultrasound findings and first- or second-trimester serum biochemical analysis. However, in the routine clinical implementation of cfDNA testing clinicians should be aware that the reported high performance of cfDNA testing may be overestimated because of the high degree of bias in certain domains of the studies included in this meta-analysis.

A positive or high-risk cfDNA result should be confirmed by invasive testing. In the case of first-trimester screening and positive cfDNA result for trisomy 21 the diagnostic test can be chorionic villous sampling. In the case of trisomies 18 and 13 a positive result should be followed by a detailed ultrasound examination and if the characteristic defects associated with these trisomies are detected then chorionic villous sampling can be carried out; if no defects are detected in the scan the preferred diagnostic test is amniocentesis to avoid an erroneous result due to placenta confined mosaicism.

A negative or low-risk cfDNA result is reassuring that the fetus is unlikely to be affected by the trisomy under investigation. The *posterior* risk for a given patient can be obtained by multiplying the *prior* risk with the negative likelihood ratios calculated in this meta-analysis; the risk for trisomies 21, 18 and 13 is reduced by a factor of 333, 56 and 100, respectively. For example, if prior screening by the combined test had shown that the risk for trisomy 21 was 1 in 100 and cfDNA testing gives a low-risk result the chance that the fetus is affected is 1 in 33,300; in contrast, if the risk for trisomy 18 from the combined test was 1 in 2 and cfDNA testing gives a low-risk result the chance that the fetus is affected is 1 in 112. Another factor that should be considered in the assessment of a cfDNA tesult is the fetal fraction;[44] however the exact methodology for incorporating fetal fraction in the calculation of the *posterior* risk depends on the precision of the assay and would vary with each company.

There are essentially two options in the clinical implementation of cfDNA analysis of maternal blood in screening for trisomies 21, 18 and 13: firstly, routine screening of the whole population and secondly, contingent screening based on the results of first-line screening by another method, preferably the first-trimester combined test. Contingent screening would lead to very high DR and very low invasive testing rate at a considerably lower cost than carrying out cfDNA testing as a first-line method of screening.[45,46] The results of the combined test would stratify the population into a very high-risk group that would best be managed by invasive testing, an intermediate-risk group that would benefit from cfDNA testing and a low-risk group that may not require any further tests for trisomies. In addition, the first-trimester scan would detect many major defects.[46]

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Figure legends

Figure 1. Flow chart for the systematic review.

Figure 2. Summary of the quality of included studies on trisomies (left) and sex chromosome aneuploidies (right) using the Quality Assessment tool for Diagnostic Accuracy Studies (QUADAS-2) checklist.

Figure 3. Funnel plots demonstrating assessment of publication bias in screening for trisomies 21, 18 and 13. A vertical pattern would indicate no publication bias whereas slope indicates the degree of bias.

Figure 4. Forest plots of failure rate of cell-free DNA test with 95% confidence intervals (CI) classified according to the method used for the cell-free DNA analysis.

Figure 5. Forest plots of sensitivity and specificity with 95% confidence intervals (CI) and weighted pooled summary statistics using bivariate random-effects regression model in assessing cell-free DNA analysis in screening for trisomy 21.

Figure 6. Forest plots of sensitivity and specificity with 95% confidence intervals (CI) and weighted pooled summary statistics using bivariate random-effects regression model in assessing cell-free DNA analysis in screening for trisomy 18.

Figure 7. Forest plots of detection sensitivity and specificity with 95% confidence intervals (CI) and weighted pooled summary statistics using bivariate random-effects regression model in assessing cell-free DNA analysis in screening for trisomy 13.

Figure 8. Forest plots of sensitivity and specificity with 95% confidence intervals (CI) and weighted pooled summary statistics using bivariate random-effects regression model in assessing cell-free DNA analysis in screening for monosomy X.

Figure 9. Forest plots of sensitivity and specificity with 95% confidence intervals (CI) and weighted pooled summary statistics using univariate random-effects model in assessing cell-free DNA analysis in screening for sex chromosome abnormalities other than monosomy X.

Figure 10. Forest plots sensitivity and specificity with 95% confidence intervals (CI) and weighted pooled summary statistics using univariate random-effects model in assessing cell-free analysis in screening for trisomy 21 in twin pregnancies.

Table 1. Summary of characteristics of the included studies.

Author	Singleton/twin gestation	Aneuploidy	N	T21	T18	T13	SCA	Outcome %	Method	GA (w)	Population
Jiang (2012)[6]	Singleton	T21/T18/T13/SCA	903	16	12	2	3	100	MPSS	- (10-34)	High-risk
Lau (2012)[7]	Singleton	T21/T18/T13/SCA	109	12	10	2	9	100	MPSS	12 (11-28)	High-risk
Nicolaides (2012)[8]	Singleton	T21/T18	1949	8	2			97	CSS	12 (11-13)	Routine
Norton (2012)[9]	Singleton	T21/T18	2969	81	38			100	CSS	16 (10-38)	High-risk
Lau (2013)[10]	Twin	T21	12	1				100	MPSS	13 (11-20)	High-risk
Liang (2013)[11]	Singleton	T21/T18/T13/SCA	406	39	13	3	8	97	MPSS	21 (11-39)	High-risk
Nicolaides (2013)[12]	Singleton	T21/T18/T13/SCA	229	25	2	1	2	100	SNP	13 (11-13)	High-risk
Song (2013)[13]	Singleton	T21/T18/T13/SCA	1741	8	2	1	3	94	MPSS	16 (11-21)	Routine
Stumm (2013)[14]	Singleton	T21/T18/T13	471	41	8	5		98	MPSS	15 (11-32)	High-risk
Verweij (2013)[15]	Singleton	T21	504	18				>96	CSS	14 (10-28)	High-risk
Bianchi (2014)[16]	Singleton	T21/T18/T13	1952	5	2	1		96	MPSS	17 (8-39)	Routine
Comas (2014)[17]	Singleton	T21	315	4				95	CSS/SNP	14 (9-23)	Mixture
Huang (2014)[18]	Twin	T21/T18	189	9	2			100	MPSS	19 (11-36)	High-risk
Pergament (2014)[19]	Singleton	T21/T18/T13	963	58	24	11		100	SNP	14 (7-40)	Mixture
Porreco (2014)[20]	Singleton	T21/T18/T13/SCA	3322	137	39	16	15	100	MPSS	17 (9-37)	High-risk
Shaw (2014)[21]	Singleton	T21/T18/T13/SCA	195	11	7	3	1	100	MPSS	> 12	Mixture
	Twin	T21/T18/T13/SCA	4		1			100	MPSS	> 12	Mixture
Benachi (2015)[22]	Singleton	T21/T18/T13	886	76	25	12		99	MPSS	15 (10-34)	High-risk
	Twin	T21/T18/T13	7	2				99	MPSS	15 (10-34)	High-risk
Ke (2015)[23]	Singleton	T21/T18/T13	2340	17	6	1		100	MPSS	> 12	High-risk
Lee (2015)[24]	Singleton	T21/T18/T13	90	5	2	1		100	MPSS	21 (8-31)	High-risk
Norton (2015)[25]	Singleton	T21/T18/T13	15841	38	10	2		92	CSS	12 (10-14)	Routine
Quezada (2015)[26]	Singleton	T21/T18/T13	2785	32	10	5		98	CSS	10 (10-11)	Routine
Song (2015)[27]	Singleton	T21/T18/T13/SCA	203	2	1	1	4	96	MPSS	9 (8-12)	High-risk
Chitty (2016)[28]	Singleton	T21/T18/T13	2301	42	?	?		94	MPSS	14 (-)	Routine
Gil (2016a)[29]	Singleton	T21/T18/T13	3633	44	21	7		98	CSS	12 (11-14)	Routine
Gil (2016b)[30]	Singleton	T21/T18/T13	54	1				94	CSS	12 (11-13)	High-risk
Hu (2016)[31]	Both*	T21/T18/T13	166572	997	257	34		88	MPSS	17 (9-36)	Mixture
Kim (2016)[32]	Singleton	T21	101	5				100	MPSS	-	High-risk
Ma (2016)[33]	Singleton	T21/T18/T13	10569	157	45	3		100	MPSS	19 (-)	High-risk
Mnyani (2016)[34]	Singleton	T21/T18/T13	80			1		90	SNP	14 (13-21)	Mixture
Oepkes (2016)[35]	Singleton	T21/T18/T13	1386	30	4	4		99	MPSS	-	High-risk
Persico (2016)[36]	Singleton	T21/T18/T13/SCA	249	36	13	5	4	100	SNP	12 (11-13)	High-risk
Qi (2016)[39]	Singleton	T21/T18/T13	2788	18	5	1		99	MPSS	19 (11-30)	High-risk
Sarno (2016)[37]	Twin	T21/T18/T13	417	8	4	1		94	CSS	11 (10-12)	Routine
Tan (2016)[38]	Twin	T21	510	4				90	MPSS	12 (11-28)	Routine
Zhang (2016)[40]	Singleton	T21/T18/SCA	87	3	1		1	100	MPSS	19 (12-32)	High-risk

Only the first author of each study is given. Gestational age (GA) is given as median (range) unless otherwise indicated. SCA, sex chromosome aneuploidy; MPSS, massively parallel shotgun sequencing; CSS, chromosome-specific sequencing; SNP, single nucleotide polymorphism based method. *It was not possible to differentiate singleton from twin pregnancies and the authors did not respond to our request for clarification.

Table 2. QUADAS-2 assessment.

Author	RISK OF BIAS				CONCERNS REGARDING APPLICABILITY		
	Patient Selection	Index Test	Reference Standard	Flow And Timing	Patient Selection	Index Test	Reference Standard
Jiang (2012)[6]	High	High	Low	High	High	High	Low
Lau (2012)[7]	High	Low	Low	Low	High	Low	Low
Nicolaides (2012)[8]	Low	Low	Low	High	Low	Low	Low
Norton (2012)[9]	High	Low	Low	High	High	Low	Low
Lau (2013)[10]	High	Low	Low	Low	High	Low	Low
Liang (2013)[11]	High	Low	Low	High	High	Low	Low
Nicolaides (2013)[12]	High	Low	Low	High	High	Low	Low
Song (2013)[13]	High	Low	High	High	Low	Low	High
Stumm (2013)[14]	Low	Low	Low	High	High	Low	Low
Verweij (2013)[15]	Low	Low	Low	High	High	Low	Low
Bianchi (2014)[16]	High	Low	Low	High	Low	Low	Low
Comas (2014)[17]	High	Low	High	High	Low	Low	High
Huang (2014)[18]	High	Low	Low	Low	High	Low	Low
Pergament (2014)[19]	High	Low	Low	High	High	Low	Low
Porreco (2014)[20]	High	Low	Low	High	High	Low	Low
Shaw (2014)[21]	High	Low	High	High	High	Low	High
Benachi (2015)[22]	High	Low	Low	High	High	Low	Low
Ke (2015)[23]	High	High	Low	High	High	High	Low
Lee (2015)[24]	Low	High	Unclear	High	High	High	Unclear
Norton (2015)[25]	Low	Low	Low	High	Low	Low	Low
Quezada (2015)[26]	High	Low	Low	High	Low	Low	Low
Song (2015)[27]	High	Low	High	High	High	Low	High
Chitty (2016)[28]	Low	Low	Low	High	Low	Low	Low
Gil (2016a)[29]	Low	Low	Low	High	Low	Low	Low
Gil (2016b)[30]	Low	Low	Low	High	High	Low	Low
Hu (2016)[31]	High	Low	Low	High	High	Low	Low
Kim (2016)[32]	High	Low	Low	Low	High	Low	Low
Ma (2016)[33]	High	Low	Low	High	High	Low	Low
Mnyani (2016)[34]	Low	Low	Low	High	High	Low	Low
Oepkes (2016)[35]	Low	Low	Low	High	High	Low	Low
Persico (2016)[36]	Low	Low	Low	High	High	Low	Low
Qi (2016)[39]	Low	Low	Low	High	High	Low	Low
Sarno (2016)[37]	Low	Low	Low	High	Low	Low	Low
Tan (2016)[38]	Low	Low	Low	High	Low	Low	Low
Zhang (2016)[40]	High	Low	High	High	High	Low	High

Only the first author of each study is given. Each study was assessed as being at high-, low- or unclear risk of bias for each domain.

Table 3. Failure to obtain a result from cell-free DNA analysis of maternal blood in screening for trisomies 21, 18 and 13 and sex chromosome aneuploidies.

Author	Method	GA (w)	Aneuploidy	Inadequate sample	Laboratory failure		
					Total	Low FF (<4%)	Assay failure
Group A: laboratory failure not reported							
Singleton pregnancies							
Ke (2015)[23]	MPSS	≥12	T21, T18, T13				
Kim (2016)[32]	MPSS	- (11-18)	T21				
Hu (2016)[31]	MPSS	17 (9-36)	T21, T18, T13				
Zhang (2016)[40]	MPSS	19 (12-32)	T21, T18, T13, SCA				
Group B: no data on low FF as reason for laboratory failure							
Singleton pregnancies							
Jiang (2012)[6]	MPSS	- (10-34)	T21, T18, T13		0/903 (0.0%)		
			SCA		1/903 (0.1%)		
Lau (2012)[7]	MPSS	12 (11-28)	T21, T18, T13, SCA		0/108 (0.0%)		
Liang (2013)[11]	MPSS	21 (11-39)	T21, T18, T13, SCA		12/435 (2.8%)		
Nicolaides (2013)[12]	SNP	13 (11-13)	T21, T18, T13, SCA		13/242 (5.4%)		
Song (2013)[13]	MPSS	16 (11-21)	T21, T18, T13, SCA		73/1916 (3.8%)		
Stumm (2013)[14]	MPSS	15 (11-32)	T21, T18, T13		32/504 (6.3%)		
Bianchi (2014)[16]	MPSS	17 (8-39)	T21, T18, T13	8/2050 (0.4%)	18/2042 (0.9%)		
Comas (2014)[17]	CSS/ SNP	14 (9-23)	T21, SCA		4/333 (1.2%)		
Porreco (2014)[20]	MPSS	17 (9-37)	T21, T18, T13	464/4170 (11.1%)	324/3700 (8.8%)		
			X analysis		372/3700 (10.1%)		
			Y analysis		452/3700 (12.2%)		
Shaw (2014)[21]	MPSS	≥ 12	T21, T18, T13, SCA	1 (0.5%)			
Benachi (2015)[22]	MPSS	15 (10-34)	T21, T18, T13		6/893 (0.7%)		
Song (2015)[27]	MPSS	9 (8-12)	T21, T18, T13, SCA	1/213 (0.5%)	0/212 (0.0%)		
Chitty (2016)[28]	MPSS	14 (-)	T21, T18, T13		94/2494 (3.7%)		
			SCA		1/177 (0.6%)		
Gil (2016a)[29]	CSS	12 (11-14)	T21, T18, T13		99/3698 (2.7%)		
Oepkes (2016)[35]	MPSS	-	T21, T18, T13		10/1390 (0.7%)		
Persico (2016)[36]	SNP	12 (11-13)	T21, T18, T13		6/250 (3.9%)		
Twin pregnancies							
Lau (2013)[10]	MPSS	13 (11-20)	T21		0/12 (0.0%)		
Huang (2014)[14]	MPSS	19 (11-36)	T21, T18		0/189 (0.0%)		
Benachi (2015)[22]	MPSS	15 (10-34)	T21, T18, T13		0/7 (0%)		
Sarno (2016)[37]	CSS	11 (10-12)	T21, T18, T13		41/438 (9.4%)		
Tan (2016)[38]	MPSS	12 (11-28)	T21		18/565 (3.2%)		
Group C: details on reason for laboratory failure							
Singleton pregnancies							
Nicolaides (2012)[8]	CSS	12 (11-13)	T21, T18	100/2149 (4.7%)	100/2049 (4.9%)	46/2049 (2.2%)	54/2049 (2.6%)
Norton (2012)[9]	CSS	16 (10-38)	T21, T18	104/4002 (2.6%)	148/3228 (4.6%)	57/3228 (1.8%)	91/3228 (2.8%)
Verweij (2013)[15]	CSS	14 (10-28)	T21	30/595 (5.0%)	16/520 (3.1%)	7/520 (1.3%)	9/520 (1.7%)
Pergament (2014)[19]	SNP	14 (7-40)	T21, T18, T13,		85/1051 (8.1%)	64/1051 (6.1%)	21/1051 (2.0%)
Lee (2015)[24]	MPSS	21 (8-31)	T21, T18, T13		1/90 (1.1%)	1/90 (1.1%)	
Norton (2015)[25]	MPSS	12 (10-14)	T21, T18, T13	384/1895 (2.0%)	488/16329 (3.0%)	192/16329 (1.2%)	213/16329 (1.3%)
Quezada (2015)[26]	CSS	10 (10-11)	T21, T18, T13	1/2905 (0.03%)	53/2905 (1.8%)	38/2905 (1.3%)	15/2905 (0.5%)
Gil (2016b)[30]	CSS	- (11-13)	T21, T18, T13		1/54 (1.9%)	1/54 (1.9%)	
Ma (2016)[33]	MPSS	19 (-)	T21, T18, T13		5/3244 (0.2%)		5/3244 (0.2%)
Mnyani (2016)[34]	SNP	14 (13-21)	T21, T18, T13		2/82 (2.4%)	1/82 (1.2%)	1/82 (1.2%)
Qi (2016)[39]	MPSS	19 (11-30)	T21, T18, T13		4/2828 (0.1%)	4/2828 (0.1%)	

Only the first author of each study is given. Gestational age (GA) is given as median (range) unless otherwise indicated. CSS, chromosome specific sequencing; FF, fetal fraction; MPSS, massively parallel shotgun sequencing; SNP, single nucleotide polymorphism-based method.

Table 4. Studies reporting on the application of cell-free DNA analysis of maternal blood in screening for trisomy 21.

Study	Trisomy 21		Non trisomy 21	
	Total	Detection rate n (%, 95% CI)	Total	False positive rate n (%, 95% CI)
Jiang (2012)[6]	16	16 (100, 79.4-100)	887	0 (0.00, 0.00-0.42)
Lau (2012)[7]	11	11 (100, 71.5-100)	97	0 (0.00, 0.00-3.73)
Nicolaides (2012)[8]	8	8 (100, 63.1-100)	1941	0 (0.00, 0.00-0.19)
Norton (2012)[9]	81	81 (100, 95.6-100)	2888	1 (0.04, 0.00-0.19)
Liang (2013)[11]	39	39 (100, 91.0-100)	367	0 (0.00, 0.00-1.00)
Nicolaides (2013)[12]	25	25 (100, 86.3-100)	204	0 (0.00, 0.00-1.79)
Song (2013)[13]	8	8 (100, 63.1-100)	1733	0 (0.00, 0.00-0.21)
Stumm (2013)[14]	41	40 (97.6, 87.2-99.9)	430	0 (0.00, 0.00-0.85)
Verweij (2013)[15]	18	17 (94.4, 72.7-99.9)	486	0 (0.00, 0.00-0.76)
Bianchi (2014)[16]	5	5 (100, 47.8-100)	1947	6 (0.31, 0.11-0.67)
Comas (2014)[17]	4	4 (100, 39.8-100)	311	0 (0.00, 0.00-1.18)
Pergament (2014)[19]	58	58 (100, 93.8-100)	905	0 (0.00, 0.00-0.41)
Porreco (2014)[20]	137	137 (100, 97.3-100)	3185	3 (0.09, 0.02-0.28)
Shaw (2014)[21]	11	11 (100, 71.5-100)	184	0 (0.00, 0.00-1.98)
Benachi (2015)[22]	74	74 (100, 95.1-100)	805	1 (0.12, 0.00-0.69)
Ke (2015)[23]	17	17 (100, 80.5-100)	2323	0 (0.00, 0.00-0.16)
Lee (2015)[24]	5	5 (100, 47.8-100)	85	0 (0.00, 0.00-4.25)
Norton (2015)[25]	38	38 (100, 90.8-100)	15803	9 (0.06, 0.03-0.11)
Quezada (2015)[26]	32	32 (100, 89.1-100)	2753	1 (0.04, 0.00-0.20)
Song (2015)[27]	2	2 (100, 15.8-100)	201	0 (0.00, 0.00-1.82)
Chitty (2016)[28]	42	42 (100, 91.6-100)	2259	1 (0.04, 0.00-0.25)
Gil (2016a)[29]	44	43 (97.7, 88.0-99.9)	3589	1 (0.03, 0.00-0.16)
Gil (2016b)[30]	1	1 (100, 25.0-100)	53	0 (0.00, 0.00-6.72)
Hu (2016)[31]	997	996 (99.9, 99.4-100)	166675	37 (0.02, 0.02-0.03)
Kim (2016)[32]	5	5 (100, 47.8-100)	96	0 (0.00, 0.00-3.77)
Ma (2016)[33]	157	157 (100, 97.7-100)	10412	2 (0.02, 0.00-0.07)
Oepkes (2016)[35]	30	29 (96.7, 82.8-99.9)	1346	2 (0.15, 0.02-0.54)
Persico (2016)[36]	36	35 (97.2, 85.5-99.9)	213	2 (0.94, 0.11-3.35)
Qi (2016)[39]	18	18 (100, 81.5-100)	2770	0 (0.00, 0.00-0.13)
Zhang (2016)[40]	3	3 (100, 29.2-100)	84	0 (0.00, 0.00-4.30)
Pooled analysis, %, (95% CI)*	99.7 (99.1-99.9)		0.04 (0.02-0.08)	
Heterogeneity assessment	1.195		0.723	
Likelihood ratio positive	2509 (1409; 4468)			
Likelihood ratio negative	0.0030 (0.0010; 0.0088)			

*Bivariate random-effects regression model; CI, confidence interval; MPSS, massively parallel shotgun sequencing; CSS, chromosome-specific sequencing; SNP, single nucleotide polymorphism based method; Var (logit accuracy), Variance of accuracy indices (logit sensitivity or logit specificity)

Table 5. Studies reporting on the application of cell-free DNA analysis of maternal blood in screening for trisomy 18.

Author	Trisomy 18		Non trisomy 18	
	Total	Detection rate n (%, 95% CI)	Total	False positive rate n (%, 95% CI)
Jiang (2012)[6]	12	12 (100, 73.5-100)	891	1 (0.11, 0.00-0.62)
Lau (2012)[7]	10	10 (100, 69.2-100)	98	0 (0.00, 0.00-3.69)
Nicolaides (2012)[8]	2	2 (100, 15.8-100)	1947	2 (0.10, 0.01-0.37)
Norton (2012)[9]	38	37 (97.4, 86.2-99.9)	2888	2 (0.07, 0.01-0.25)
Liang (2013)[11]	13	13 (100, 75.3-100)	393	0 (0.00, 0.00-0.93)
Nicolaides (2013)[12]	3	3 (100, 29.2-100)	226	0 (0.00, 0.00-1.62)
Song (2013)[13]	2	2 (100, 15.8-100)	1739	1 (0.06, 0.00-0.32)
Stumm (2013)[14]	8	8 (100, 63.1-100)	463	1 (0.22, 0.01-1.20)
Bianchi (2014)[16]	2	2 (100, 15.8-100)	1950	3 (0.15, (0.03-0.45)
Pergament (2014)[19]	24	24 (100, 85.8-100)	938	0 (0.00, 0.00-0,39)
Porreco (2014)[20]	39	36 (92.3, 79.1-98.4)	3283	0 (0.00, 0.00-0.11)
Shaw (2014)[21]	7	7 (100, 59.0-100)	188	0 (0.00, 0.00-1.94)
Benachi (2015)[22]	25	22 (88.0, 68.8-97.5)	854	1 (0.12, 0.00-0.65)
Ke (2015)[23]	6	6 (100, 54.1-100)	2334	0 (0.00, 0.00-0.16)
Lee (2015)[24]	2	2 (100, 15.8-100)	88	0 (0.00, 0.00-4.11)
Norton (2015)[25]	10	9 (90.0, 55.5-99.8)	5831	1 (0.02, 0.00-0.10)
Quezada (2015)[26]	10	9 (90.0, 55.5-99.8)	2775	5 (0.18, 0.06-0.42)
Song (2015)[27]	1	1 (100.0, 2.50-100.0)	202	0 (0.00, 0.00-1.81)
Gil (2016a)[29]	21	21 (100, 83.9-100)	3612	4 (0.11, 0.03-0.28)
Hu (2016)[31]	257	256 (99.6, 97.9-100)	166315	38 (0.02, 0.02-0.03)
Ma (2016)[33]	45	45 (100, 92.1-100)	10527	2 (0.02, 0.00-0.07)
Oepkes (2016)[35]	4	4 (100, 39.8-100)	1372	1 (0.07, 0.00-0.41)
Persico (2016)[36]	13	13 (100, 76.3-100)	236	0 (0.00, 0.00-1.55)
Qi (2016)[39]	5	5 (100, 47.8-100)	2783	1 (0.04, 0.00-0.20)
Zhang (2016)[40]	1	1 (100, 25.0-100)	86	0 (0.00, 0.00-4.20)
Pooled analysis, %, (95% CI)*	98.2 (95.5-99.2)		0.05 (0.03-0.07)	
Heterogeneity assessment	0.905		0.299	
Likelihood ratio positive	2122 (1350; 3337)			
Likelihood ratio negative	0.018 (0.008; 0.045)			

*Bivariate random-effects regression model; CI, confidence interval; MPSS, massively parallel shotgun sequencing; CSS, chromosome-specific sequencing; SNP, single nucleotide polymorphism based method; Var (logit accuracy), Variance of accuracy indices (logit sensitivity or logit specificity)

Table 6. Studies reporting on the application of cell-free DNA analysis of maternal blood in screening for trisomy 13.

Author	Trisomy 13		Non trisomy 13	
	Total	Detection rate n (%, 95% CI)	Total	False positive rate n (%, 95% CI)
Jiang (2012)[6]	2	2 (100, 15.8-100)	901	0 (0.00, 0.00-0.41)
Lau (2012)[7]	2	2 (100, 15.8-100)	106	0 (0.00, 0.00-3.42)
Liang (2013)[11]	3	3 (100, 29.2-100)	403	1 (0.25, 0.01-1.38)
Nicolaides (2013)[12]	1	1 (100, 2.5-100)	228	0 (0.00, 0.00-1.61)
Song (2013)[13]	1	1 (100, 2.5-100)	1740	0 (0.00, 0.00-0.21)
Stumm (2013)[14]	5	5 (100, 47.8-100)	466	0 (0.00, 0.00-0.79)
Bianchi (2014)[16]	1	1 (100, 2.5-100)	1913	3 (0.16, 0.03-0.46)
Pergament (2014)[19]	11	11 (100, 71.5-100)	953	0 (0.00, 0.00-0.39)
Porreco (2014)[20]	16	14 (87.5, 61.7-98.5)	3306	0 (0.00, 0.00-0.11)
Shaw (2014)[21]	3	3 (100, 29.2-100)	192	0 (0.00, 0.00-1.90)
Benachi (2015)[22]	12	12 (100, 73.5-100)	867	1 (0.12, 0.00-0.64)
Ke (2015)[23]	1	1 (100, 2.5-100)	2339	0 (0.00, 0.00-0.16)
Lee (2015)[24]	1	0 (0.00, 0.00-97.5)	89	89 (0.00, 0.00-4.06)
Norton (2015)[25]	2	2 (100, 15.8-100)	11183	2 (0.02, 0.00-0.06)
Quezada (2015)[26]	5	2 (40.0, 52.8-85.3)	2780	2 (0.07, 0.01-0.26)
Song (2015)[27]	1	1 (100.0, 2.5-100.0)	202	0 (0.00, 0.00-1.81)
Gil (2016a)[29]	4	2 (50.0, 6.8-93.2)	3629	4 (0.11, 0.03-0.28)
Hu (2016)[31]	34	34 (100, (89.7-100)	166538	72 (0.04, 0.03-0.05)
Ma (2016)[33]	3	3 (100, 29.2-100)	10566	0 (0.00, 0.00-0.03)
Mnyani (2016)[34]	1	1 (100, 2.5-100)	79	0 (0.00, 0.00-4.56)
Oepkes (2016)[35]	4	4 (100, 39.8-100)	1372	2 (0.15, 0.02-0.53)
Persico (2016)[36]	5	5 (100, 47.8-100)	244	0 (0.00, 0.00-1.50)
Qi (2016)[39]	1	1 (100, 2.5-100)	2787	1 (0.04, 0.00-0.20)
Pooled analysis, %, (95% CI)*	99.0 (65.8-100)		0.04 (0.02-0.07)	
Heterogeneity assessment	8.818		0.459	
Likelihood ratio positive	2819 (1440; 5521)			
Likelihood ratio negative	0.0100 (0.0002; 0.4940)			

*Bivariate random-effects regression model; CI, confidence interval; MPSS, massively parallel shotgun sequencing; CSS, chromosome-specific sequencing; SNP, single nucleotide polymorphism based method; Var (logit accuracy), Variance of accuracy indices (logit sensitivity or logit specificity)

Table 7. Studies reporting on the application of cell-free DNA analysis of maternal blood in screening for monosomy X.

Author	Monosomy X		Non Monosomy X	
	Total	Detection rate n (%, 95% CI)	Total	False positive rate n (%, 95% CI)
Jiang (2012)[6]	3	3 (100.0, 29.2-100.0)	899	1 (0.11, 0.00-0.62)
Lau (2012)[7]	8	8 (100.0, 63.1-100.0)	100	0 (0.00, 0.00-3.62)
Liang (2013)[11]	5	5 (100.0, 47.8-100.0)	401	1 (0.25, 0.01-1.38)
Nicolaides (2013)[12]	2	2 (100.0, 15.8-100.0)	227	0 (0.00, 0.00-1.61)
Song (2013)[13]	3	2 (66.7, 9.4-99.2)	1737	0 (0.00, 0.00-0.21)
Comas (2014)[17]	0	-	315	1 (0.32, 0.01-1.76)
Porreco (2014)[20]	9	9 (100.0, 66.4-100.0)	3269	11 (0.34, 0.17-0.60)
Shaw (2014)[21]	3	3 (100.0, 29.2-100.0)	192	0 (0.00, 0.00-1.90)
Song (2015)[27]	0	-	203	1 (0.49, 0.01-2.71)
Persico (2016)[36]	3	2 (66.7, 9.4-99.2)	248	1 (0.40, 0.01-2.23)
Zhang (2016)[40]	0	-	86	0 (0.00, 0.00-4.20)
Pooled analysis, %, (95% CI)*	95.8 (70.3-99.5)		0.14 (0.05-0.38)	
Heterogeneity assessment	1.329		0.409	
Likelihood ratio positive	694 (253; 1902)			
Likelihood ratio negative	0.0421 (0.0048; 0.3688)			

*Bivariate random-effects regression model; CI, confidence interval; MPSS, massively parallel shotgun sequencing; CSS, chromosome-specific sequencing; SNP, single nucleotide polymorphism based method; Var (logit accuracy), Variance of accuracy indices (logit sensitivity or logit specificity)

Table 8. Studies reporting on the application of cell-free DNA analysis of maternal blood in screening for sex chromosome abnormalities other than monosomy X.

Author	47,XXX; 47,XXY; 47,XYY		Non SCA	
	Total	Detection rate n (%, 95% CI)	Total	False positive rate n (%, 95% CI)
Jiang (2012)[6]	3	3 (100.0, 29.2-100.0)	896	0 (0.00, 0.00-0.41)
Lau (2012)[7]	1	1 (100.0, 2.5-100.0)	99	0 (0.00, 0.00-3.66)
Liang (2013)[11]	3	3 (100.0, 29.2-100.0)	398	1 (0.25, 0.01-1.39)
Porreco (2014)[20]	6	6 (100.0, 54.1-100.0)	3263	5 (0.15, 0.05-0.36)
Shaw (2014)[21]	1	1 (100.0, 2.5-100.0)	191	0 (0.00, 0.00-1.91)
Song (2015)[27]	1	0 (0.0, 0.0-97.5)	202	0 (0.00, 0.00-1.81)
Persico (2016)[36]	1	1 (100.0, 2.5-100.0)	248	0 (0.00, 0.00-1.48)
Zhang (2016)[40]	1	1 (100.0, 2.5-100.0)	86	0 (0.00, 0.00-4.20)
Pooled analysis, %, (95% CI)*	100 (83.6-100)		0.003 (0-0.07)	
I^2 statistic (%)	0%		0%	

*Univariate random-effects regression model; CI, confidence interval; MPSS, massively parallel shotgun sequencing; CSS, chromosome-specific sequencing; SNP, single nucleotide polymorphism based method; SCA, sex chromosome abnormality

Table 9. Studies reporting on the application of cell-free DNA analysis of maternal blood in screening for trisomy 21 in twin pregnancies.

Author	Trisomic		Non trisomic	
	Total	**n (%, 95% CI)**	**Total**	**n (%, 95% CI)**
Lau (2013)[10]	1	1 (100, 2.5-100)	11	0 (0.0, 0.0-28.5)
Huang (2014)[14]	9	9 (100, 66.4-100)	180	0 (0.00, 0.00-2.03)
Benachi (2015)[22]	2	2 (100, 15.8-100)	5	0 (0.00, 0.00-52.18)
Sarno (2016)[37]	8	8 (100, 63.1-100)	409	0 (0.00, 0.00-0.90)
Tan (2016)[38]	4	4 (100, 39.8-100)	506	0 (0.00, 0.00-0.73)
Pooled analysis, %, (95% CI)*	100 (95.2-100)		0 (0-0.003)	
I^2 statistic (%)	0%		0%	

*Univariate random-effects regression model; CI, confidence interval; MPSS, massively parallel shotgun sequencing; CSS, chromosome-specific sequencing

Table 10. Subgroup analysis of cell-free DNA testing for trisomies 21 and 18 in singleton pregnancies.

	Studies	Sensitivity (95% CI)	p value *	Specificity (95% CI)	p value * *
TRISOMY 21					
Method					
MPSS	19	0.9981 (0.9913; 0.9996)	-	0.9996 (0.9992; 0.9998)	-
CSS	7	0.9910 (0.9647; 0.9977)	0.526	0.9996 (0.9992; 0.9998)	0.361
SNP[#]	3	0.9973 (0.9715; 1.0000)	0.789	0.9991 (0.9914; 1.0000)	0.102
1st trimester					
No	21	0.9978 (0.9908; 0.9995)	0.590	0.9996 (0.9993; 0.9998)	0.765
Yes	9	0.9912 (0.9656; 0.9978)		0.9994 (0.9991; 0.9997)	
Population					
High-risk	19	0.9963 (0.9819; 0.9992)	-	0.9996 (0.9990; 0.9998)	-
Mixture	4	0.9991 (0.9933; 0.9999)	0.102	0.9998 (0.9997; 0.9998)	0.198
Routine	7	0.9957 (0.9321; 0.9997)	0.984	0.9995 (0.9987; 0.9998)	0.146
TRISOMY 18					
Method**					
MPSS	17	0.9868 (0.9412; 0.9971)	-	0.9997 (0.9994; 0.9998)	-
CSS	5	0.9634 (0.8898; 0.9885)	0.789	0.9992 (0.9984; 0.9996)	0.848
SNP[#]	3	1.0000 (0.9736; 1.0000)	0.601	1.0000 (0.9990; 1.0000)	0.746
1st trimester					
No	18	0.9862 (9.9521; 0.9961)	0.302	0.9997 (0.9994; 0.9998)	0.465
Yes	7	0.9672 (0.8717; 0.9922)		0.9992 (0.9983; 0.9996)	
Population					
High-risk	16	0.9805 (0.9063; 0.9962)	-	0.9997 (0.9993; 0.9998)	-
Mixture[#]	2	1.0000 (0.9673; 1.0000)	0.008	1.0000 (0.9988; 1.0000)	0.013
Routine	6	0.9591 (0.8366; 0.9908)	0.414	0.9991 (0.9984; 0.9907)	0.802

MPSS, massively parallel shotgun sequencing; CSS, chromosome-specific sequencing.

* relative sensitivity; ** relative specificity; [#]random univariate model