



# Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis

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**KEYWORDS:** cell-free fetal DNA; fetal aneuploidy; non-invasive prenatal testing; trisomy 13; trisomy 18; trisomy 21; Turner syndrome

## 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.

**Methods** 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 4 January 2015.

**Results** In total, 37 relevant studies were identified and these were used for the meta-analysis on the performance of cfDNA testing in screening for aneuploidies. These studies reported cfDNA results in relation to fetal karyotype from invasive testing or clinical outcome. Weighted pooled detection rates (DR) and false-positive rates (FPR) in singleton pregnancies were 99.2% (95% CI, 98.5–99.6%) and 0.09% (95% CI, 0.05–0.14%), respectively, for trisomy 21, 96.3% (95% CI, 94.3–97.9%) and 0.13% (95% CI, 0.07–0.20) for trisomy 18, 91.0% (95% CI, 85.0–95.6%) and 0.13% (95% CI, 0.05–0.26%) for trisomy 13, 90.3% (95% CI, 85.7–94.2%) and 0.23% (95% CI, 0.14–0.34%) for monosomy X and 93.0% (95% CI, 85.8–97.8%) and 0.14% (95% CI, 0.06–0.24%) for sex chromosome aneuploidies other than monosomy X. For twin pregnancies, the DR for trisomy 21 was 93.7% (95% CI, 83.6–99.2%) and the FPR was 0.23% (95% CI, 0.00–0.92%).

**Conclusion** Screening for trisomy 21 by analysis of cfDNA in maternal blood is superior to that of all other traditional methods of screening, with higher DR and lower FPR. The performance of screening for trisomies 18 and 13 and sex chromosome aneuploidies is considerably worse than that for trisomy 21. Copyright © 2015 ISUOG. Published by John Wiley & Sons Ltd.

## INTRODUCTION

Several studies in the last 4 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 a previous meta-analysis<sup>1</sup>, we reported the results from studies published between January 2011 and 20 December 2013. The objective of this meta-analysis was to update the results, with inclusion of studies that were published up to 4 January 2015.

## METHODS

### Literature search and study selection

Searches of PubMed, EMBASE and The Cochrane Library were performed, with a restriction to English-language publications, to identify all peer-reviewed articles published on clinical validation or implementation of maternal cfDNA testing in screening for aneuploidies. The search period was from January 2011, when the first such study was published<sup>2</sup>, to 4 January 2015. A list of relevant citations was generated from these databases using the following search terms: 'maternal blood cfDNA', 'non-invasive prenatal detection', 'noninvasive prenatal diagnosis' or 'non invasive prenatal diagnosis'.

The abstracts of citations were examined by two reviewers (M.M.G., R.R.) to identify all potentially relevant articles, which were then examined in full-text form. 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 a third reviewer (K.H.N.).

The inclusion criteria were peer-reviewed study 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

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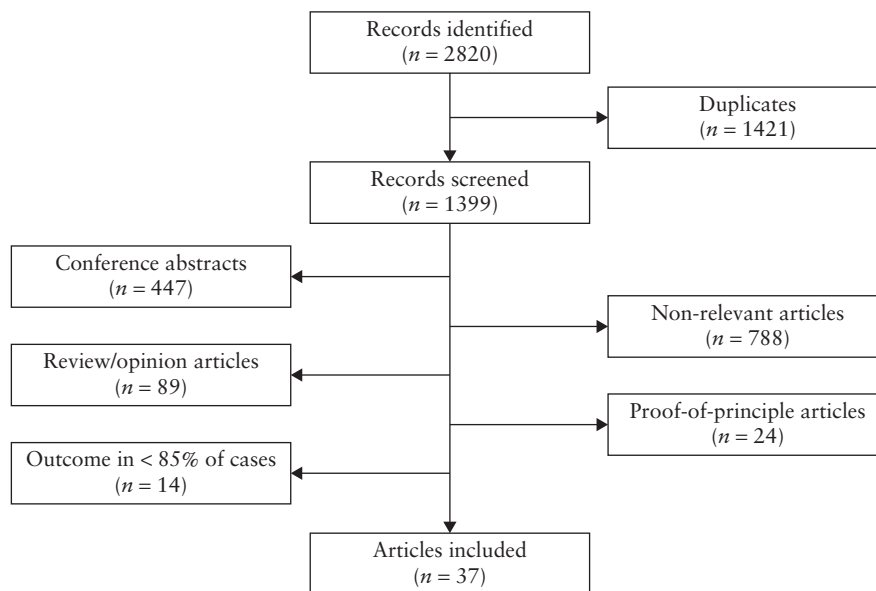


Figure 1 Flowchart summarizing selection of studies for inclusion in the systematic review.

85% of the study population. 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 detection (DR) and false-positive (FPR) rates for non-mosaic trisomies 21,18 and 13 and sex chromosome aneuploidies were 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 in which 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. In tables in which there was a zero in any cell, Haldane correction was used, which added 0.5 to each count in the table to allow for estimation of variance and pooled effects.

Meta-analysis of extracted data was carried out in two steps: first, summary statistics with 95% CIs were derived for each study and, second, individual study statistics were combined to obtain a pooled summary estimate, which was calculated as a weighted average of the individual study estimates. The pooled summary statistics were estimated using both fixed-effects (inverse variance) and random-effects (DerSimonian-Laird) models.

Assessment of quality, heterogeneity between studies and estimation of bias were carried out as described previously<sup>1</sup>.

The statistical software package StatsDirect version 2.7.9 (StatsDirect Ltd, Cheshire, UK) was used for data analysis.

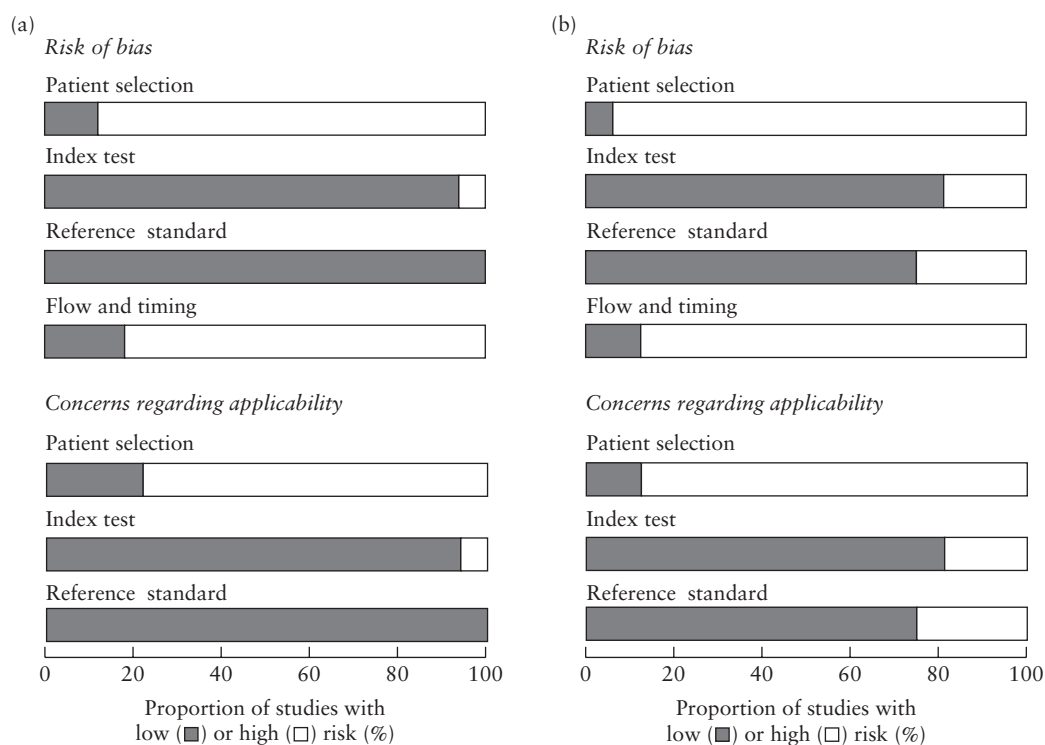
## RESULTS

### Data sources

The search identified 1399 potentially relevant citations (Figure 1). The following groups were excluded: conference abstracts rather than peer-reviewed papers ( $n = 447$ ), non-relevant publications ( $n = 788$ ), review articles or opinions ( $n = 89$ ), proof-of-principle studies reporting laboratory techniques, rather than clinical validation of a predefined method of maternal blood cfDNA analysis ( $n = 24$ )<sup>3–26</sup> and studies on clinical implementation of cfDNA testing in screening for aneuploidies in which pregnancy outcome data were provided for fewer than 85% of the study population<sup>27–40</sup> ( $n = 14$ ). One study had been included in our previous meta-analysis<sup>1</sup>, but, on further assessment for this analysis, it has been reclassified as a proof-of-principle study, as acknowledged by the authors<sup>12</sup>.

In total, 37 relevant studies were identified<sup>2,41–76</sup> and these were used for the meta-analysis on the performance of cfDNA testing in screening for aneuploidies. These studies reported cfDNA results in relation to fetal karyotype from invasive testing or clinical outcome.

In three of the 37 studies, some of the maternal blood samples for the cfDNA analysis were obtained after the invasive test<sup>54,63,71</sup>. In 27 studies, it was stated explicitly<sup>2,41–47,49–52,55–59,61,62,65,68–70,72–74,76</sup> and in two it was assumed on the basis of the described methodology<sup>64,75</sup> that, if an invasive test was carried out, the samples for cfDNA analysis were obtained before the invasive test. In five studies, it was uncertain if invasive testing was performed before or after maternal blood sampling for the cfDNA test<sup>48,53,60,66,67</sup>.



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

### Methodological quality of the selected studies

The methodological quality of the selected studies, assessed by the Quality Assessment tool for Diagnostic Accuracy Studies (QUADAS-2)<sup>77</sup>, 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 studies were assessed separately for the trisomies and the sex chromosome aneuploidies.

#### Risk of bias

The first domain relates to patient selection. 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 and any exclusions were appropriate; case-control studies were considered to be at high risk of bias. The following studies were classified as being at high risk of bias either because the samples were not stated explicitly to have been consecutive or selected at random<sup>2,41,42,44,48,49,51,53,56–61,63–66,68,69,71–73,75</sup> or because a case-control design was used<sup>43,45–47,52,54,55,67,70</sup>. Only four studies were classified as being at low risk of bias<sup>50,62,74,76</sup>.

The second domain relates to the index test. 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. The risk of bias was considered to be low in all papers that stated explicitly that the cfDNA test was performed without prior knowledge of fetal karyotype or

outcome. In four studies this was assumed to be the case, but, because it was not stated in the paper, we recorded a high risk of bias<sup>2,48,60,68</sup>.

The third domain relates to the reference standard. 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 and 13, we accepted this to be true if the diagnosis was based on prenatal or postnatal karyotyping, in the case of affected fetuses, or on 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, in four studies, the risk of bias was considered to be high because the assumption of normal karyotype was based on clinical examination at birth rather than on karyotyping<sup>61,64,73,76</sup>. Unlike the situation with trisomies 21, 18 and 13, neonates with sex chromosome aneuploidies are often phenotypically normal. Consequently, studies that do not involve karyotyping of the whole population will inevitably underestimate the true prevalence of these abnormalities and overestimate the potential sensitivity of a prenatal screening test.

The fourth domain relates to flow and timing. 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 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. Only six studies fulfilled the above two conditions and were classified as being at low risk of bias<sup>2,49,55,56,66,69</sup>. All other 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.

### Concerns regarding applicability

In the context of screening for fetal aneuploidies by cfDNA analysis of maternal blood, there would be concern regarding applicability to screening in the general population if the test in the studies included in the meta-analysis was carried out in pregnancies identified as being at high risk for aneuploidies by prior screening with another method.

In terms of the first domain on patient selection, only the five studies that were performed in a general population were classified as being at low risk of concerns regarding applicability<sup>50,61,63,64,75</sup>. In terms of the second domain, on index test, all studies classified as being at low risk of bias were also considered to be at low risk of concerns regarding applicability; there were only four papers classified as being at high risk<sup>2,48,60,68</sup>. 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 being at high risk of concerns regarding applicability<sup>61,64,73,76</sup>.

### Method of analyzing samples

The studies included in the meta-analysis used one of three methods for analysis of cfDNA in maternal blood: massively parallel shotgun sequencing (technique described in references<sup>78,79</sup>), chromosome-selective sequence analysis (technique described in references<sup>9,53</sup>) or single nucleotide polymorphism-only-based analysis (technique described in references<sup>12,80</sup>). 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 the meta-analysis were retrospective, using stored samples from pregnancies with known outcome<sup>45,54,55,60,65,66,68,70</sup>, or prospective, using mainly samples from high-risk pregnancies undergoing invasive testing<sup>42–44,46–49,51–53,56–59,62,67,69,71–74,76</sup>; two were both<sup>2,41</sup>.

Only five of the studies reported on the clinical implementation of cfDNA testing in routine screening for trisomies in the general population<sup>50,61,63,64,75</sup>. The first<sup>50</sup> examined stored plasma samples from 2049 singleton

pregnancies that underwent combined screening at 11–13 weeks' gestation and had known pregnancy outcome. Results were obtained from cfDNA testing in 1949 (95.1%) pregnancies and all 10 cases of trisomy 21 or 18 were correctly identified, with a FPR of 0.1%

In the second study<sup>61</sup>, cfDNA testing was performed prospectively in 1916 singleton pregnancies at a median gestational age of 16 (range, 11–21) weeks. The test did not provide a result in 3.8% of cases and there was loss to follow-up in 5.8% of cases. Of the 1741 pregnancies with cfDNA results and outcome data, the test correctly identified all 11 cases of trisomy 21, 18 or 13, with a FPR of 0.06%.

In the third study<sup>63</sup>, cfDNA testing was performed prospectively in 2042 singleton pregnancies at 17 (range, 8–39) weeks. The test did not provide a result in 0.9% of cases and there was loss to follow-up in 3.5% of cases. Of the 1952 pregnancies with cfDNA results and outcome data, the test correctly identified all seven cases of trisomy 21 or 18, with a FPR of 0.5%.

In the fourth study<sup>64</sup>, cfDNA testing was performed prospectively in 333 singleton pregnancies at 14 (range, 9–23) weeks. The test did not provide a result in 1.2% of cases and there was no follow-up in 5.5% of cases. Of the 315 pregnancies with cfDNA results and outcome data, the test correctly identified all four cases of trisomy 21, with a FPR of 0.0%.

In the fifth study<sup>75</sup>, cfDNA testing was performed prospectively in 2905 singleton pregnancies at 11–13 weeks. The test did not provide a result in 1.9% of cases and there was loss to follow-up in 2.3% of cases. Of the 2785 pregnancies with cfDNA results and outcome data, the test correctly identified all 32 cases with trisomy 21, nine of 10 with trisomy 18 and two of five with trisomy 13, with FPRs of 0.04%, 0.19% and 0.07%, respectively.

### 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: first, problems with blood collection and transportation of the samples to the laboratory, including inadequate blood volume, hemolysis, incorrect labeling of tubes and delay in arrival to the laboratory; second, low fetal fraction (usually below 4%); and third, assay failure for a variety of reasons, including failed DNA extraction, amplification or sequencing.

Data on the no-result rate from the studies included in the meta-analysis are summarized in Table 1. Data relating to blood collection and transportation of the samples were provided by 11 of the studies and the reported rates ranged from 0.03% to 11.1%. Data on failure to obtain results for samples that were analyzed were provided by 35 of the studies and the reported rates ranged from 0.0% to 12.2%. In 11 of these 35 studies, further details were given, with the reason for failure being low fetal fraction and the reported rates ranged from 0.5% to 6.1%.

**Table 1** Failure to obtain a result from cell-free DNA analysis of maternal blood in screening for trisomies (T) 21, 18 and 13 and sex chromosome aneuploidies (SCA)

Study	Method	GA (weeks)	Aneuploidy	Inadequate sample (n (%))	Laboratory failure (n (%))		
					Total	Low FF (< 4%)	Assay failure
<i>Laboratory failure not reported</i>							
Singleton pregnancy							
Shaw (2013) <sup>73</sup>	MPSS	> 12	T21, T18, T13, SCA				
Twin pregnancy							
Canick (2012) <sup>47</sup>	MPSS	14 (10–18)	T21, T13				
<i>No data on low FF as reason for laboratory failure</i>							
Singleton pregnancy							
Chen (2011) <sup>2</sup>	MPSS	—	T18, T13		0/289 (0.0)		
Chiu (2011) <sup>41</sup>	MPSS	13 (—)	T21	46/810 (5.7)	11/764 (1.4)		
Sehnert (2011) <sup>44</sup>	MPSS	15 (10–28)	T21, T18 SCA		1/47 (2.1) 1/47 (2.1)		
Ashoor (2012) <sup>45</sup>	CSS	12 (11–13)	T21, T18	25/425 (5.9)	3/400 (0.8)		
Jiang (2012) <sup>48</sup>	MPSS	— (10–34)	T21, T18, T13 SCA		0/903 (0.0) 1/903 (0.1)		
Lau (2012) <sup>49</sup>	MPSS	12 (11–28)	T21, T18, T13, SCA		0/108 (0.0)		
Palomaki (2012) <sup>52</sup>	MPSS	14 (9–22)	T21, T18, T13		17/1988 (0.9)		
Sparks (2012) <sup>53</sup>	CSS	18 (11–36)	T21, T18		8/338 (2.4)		
Ashoor (2013) <sup>54</sup>	CSS	12 (11–13)	T13		62/2167 (2.9)		
Guex (2013) <sup>55</sup>	MPSS	12 (11–13)	T21, T18, T13, SCA		0/276 (0.0)		
Liang (2013) <sup>57</sup>	MPSS	21 (11–39)	T21, T18, T13, SCA		12/435 (2.8)		
Mazloom (2013) <sup>58</sup>	MPSS	— (10–20)	SCA		116/1975 (5.9)		
Nicolaides (2013) <sup>59</sup>	SNP	13 (11–13)	T21, T18, T13, SCA		13/242 (5.4)		
Samango-Sprouse (2013) <sup>60</sup>	SNP	13 (9–36)	SCA		14/201 (7.0)		
Song (2013) <sup>61</sup>	MPSS	16 (11–21)	T21, T18, T13, SCA		73/1916 (3.8)		
Bianchi (2014) <sup>63</sup>	MPSS	17 (8–39)	T21, T18, T13	8/2050 (0.4)	18/2042 (0.9)		
Comas (2014) <sup>64</sup>	CSS/ SNP	14 (9–23)	T21, T18, T13, SCA		4/333 (1.2)		
Hooks (2014) <sup>68</sup>	CSS	15 (10–34)	SCA		18/432 (4.2)		
Porreco (2014) <sup>72</sup>	MPSS	17 (9–37)	T21, T18, T13 X analysis Y analysis	464/4170 (11.1)	324/3700 (8.8) 372/3700 (10.1) 452/3700 (12.2)		
Stumm (2014) <sup>74</sup>	MPSS	15 (11–32)	T21, T18, T13		32/504 (6.3)		
Song (2015) <sup>76</sup>	MPSS	9 (8–12)	T21, T18, T13, SCA	1/213 (0.5)	0/212 (0.0)		
Twin pregnancy							
Lau (2013) <sup>56</sup>	MPSS	13 (11–20)	T21		0/12 (0.0)		
Grömminger (2014) <sup>66</sup>	MPSS	15 (10–18)	T21		0/56 (0.0)		
Huang (2014) <sup>69</sup>	MPSS	19 (11–36)	T21, T18		0/189 (0.0)		
<i>Details given on reason for laboratory failure</i>							
Singleton pregnancy							
Ehrich (2011) <sup>42</sup>	MPSS	16 (8–36)	T21	13/480 (2.7)	18/467 (3.9)	7/467 (1.5)	11/467 (2.4)
Palomaki (2011) <sup>43</sup>	MPSS	15 (8–21)	T21		13/1696 (0.8)	9/1696 (0.5)	4/1696 (0.2)
Bianchi (2012) <sup>46</sup>	MPSS	15 (10–23)	T21, T18, T13 SCA	2/534 (0.4)	30/532 (5.6) 65/532 (12.2)	16/532 (3.0) 16/532 (3.0)	14/532 (2.6) 49/532 (9.2)
Nicolaides (2012) <sup>50</sup>	CSS	12 (11–13)	T21, T18	100/2149 (4.7)	100/2049 (4.9)	46/2049 (2.2)	54/2049 (2.6)
Norton (2012) <sup>51</sup>	CSS	16 (10–38)	T21, T18	104/4002 (2.6)	148/3228 (4.6)	57/3228 (1.8)	91/3228 (2.8)
Verweij (2013) <sup>62</sup>	CSS	14 (10–28)	T21	30/595 (5.0)	16/520 (3.1)	7/520 (1.3)	9/520 (1.7)
Hall (2014) <sup>67</sup>	SNP	16 (12–22)	T13		4/68 (5.9)	4/68 (5.9)	
Nicolaides (2014) <sup>70</sup>	CSS	12 (11–13)	SCA		5/177 (2.8)	4/177 (2.3)	1/177 (0.6)
Pergament (2014) <sup>71</sup>	SNP	14 (7–40)	T21, T18, T13, SCA		85/1051 (8.1)	64/1051 (6.1)	21/1051 (2.0)
Quezada (2015) <sup>75</sup>	CSS	10 (10–11)	T21, T18, T13	1/2905 (0.03)	53/2905 (1.8)	38/2905 (1.3)	15/2905 (0.52)
Twin pregnancy							
del Mar Gil (2014) <sup>65</sup>	CSS	13 (12–13)	T21, T18, T13		15/207 (7.2)	11/207 (5.3)	4/207 (1.9)

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.

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 or gestational age at sampling. However, findings from the four studies that reported the no-result rate separately for trisomies and sex chromosome aneuploidies<sup>44,46,48,72</sup> suggest that the rate for the latter is increased; the rate was 6.9% (355 of 5182) for trisomies and 17.2% (891 of 5182) 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 (Cochran's Q and  $I^2$  statistic) are provided in Tables 2–7 and illustrated in Figures 4–9. The publication bias of the studies is also given in Tables 2–7 (Egger's bias value) and assessed graphically using funnel plots in Figure 3.

#### Trisomy 21

A total of 24 studies reported on the performance of screening by cfDNA analysis for trisomy 21, in a combined

total of 1051 trisomy-21 and 21 608 non-trisomy-21 singleton pregnancies (Table 2). Among individual studies, the DR varied between 94.4% and 100% and the FPR varied between 0% and 2.05%. The pooled weighted DR and FPR were 99.2% (95% CI, 98.5–99.6%) and 0.09% (95% CI, 0.05–0.14%), respectively.

#### Trisomy 18

A total of 21 studies reported on the performance of screening by cfDNA analysis for trisomy 18, in a combined total of 389 trisomy-18 and 21 306 non-trisomy-18 singleton pregnancies (Table 3). In individual studies, the DR varied between 90.0% and 100% and the FPR varied between 0% and 1.98%. The pooled weighted DR and FPR were 96.3% (95% CI, 94.3–97.9%) and 0.13% (95% CI, 0.07–0.20), respectively.

#### Trisomy 13

A total of 18 studies reported on the performance of screening by cfDNA analysis for trisomy 13, in a combined total of 139 trisomy-13 and 18 059 non-trisomy-13 singleton pregnancies (Table 4). In individual studies, the DR varied between 40.0% and 100% and the FPR varied between 0% and 1.14%. The pooled weighted DR and

**Table 2** Studies reporting on the application of cell-free DNA analysis of maternal blood in screening for trisomy 21 in singleton pregnancy

Study	Method	GA (weeks)	Trisomy 21		Non-trisomy 21	
			Total (n)	Detection (n (%), 95% CI)	Total (n)	False positive (n (%), 95% CI)
Chiu (2011) <sup>41</sup>	MPSS	13 (—)	86	86 (100, 95.8–100)	146	3 (2.05, 0.43–5.89)
Ehrich (2011) <sup>42</sup>	MPSS	16 (8–36)	39	39 (100, 91.0–100)	410	1 (0.24, 0.01–1.35)
Palomaki (2011) <sup>43</sup>	MPSS	15 (8–21)	212	209 (98.6, 95.9–99.7)	1471	3 (0.20, 0.04–0.60)
Sehnert (2011) <sup>44</sup>	MPSS	15 (10–28)	13	13 (100, 75.3–100)	34	0 (0.00, 0.00–10.28)
Ashoor (2012) <sup>45</sup>	CSS	12 (11–13)	50	50 (100, 92.9–100)	347	0 (0.00, 0.00–1.06)
Bianchi (2012) <sup>46</sup>	MPSS	15 (10–23)	89	89 (100, 95.9–100)	404	0 (0.00, 0.00–0.91)
Jiang (2012) <sup>48</sup>	MPSS	— (10–34)	16	16 (100, 79.4–100)	887	0 (0.00, 0.00–0.42)
Lau (2012) <sup>49</sup>	MPSS	12 (11–28)	11	11 (100, 71.5–100)	97	0 (0.00, 0.00–3.73)
Nicolaidis (2012) <sup>50</sup>	CSS	12 (11–13)	8	8 (100, 63.1–100)	1941	0 (0.00, 0.00–0.19)
Norton (2012) <sup>51</sup>	CSS	16 (10–38)	81	81 (100, 95.6–100)	2888	1 (0.04, 0.00–0.19)
Sparks (2012) <sup>53</sup>	CSS	18 (11–36)	36	36 (100, 90.3–100)	131	0 (0.00, 0.00–2.78)
Guex (2013) <sup>55</sup>	MPSS	12 (11–13)	30	30 (100, 88.4–100)	146	0 (0.00, 0.00–2.50)
Liang (2013) <sup>57</sup>	MPSS	21 (11–39)	39	39 (100, 91.0–100)	367	0 (0.00, 0.00–1.00)
Nicolaidis (2013) <sup>59</sup>	SNP	13 (11–13)	25	25 (100, 86.3–100)	204	0 (0.00, 0.00–1.79)
Song (2013) <sup>61</sup>	MPSS	16 (11–21)	8	8 (100, 63.1–100)	1733	0 (0.00, 0.00–0.21)
Verweij (2013) <sup>62</sup>	CSS	14 (10–28)	18	17 (94.4, 72.7–99.9)	486	0 (0.00, 0.00–0.76)
Bianchi (2014) <sup>63</sup>	MPSS	17 (8–39)	5	5 (100, 47.8–100)	1947	6 (0.31, 0.11–0.67)
Comas (2014) <sup>64</sup>	CSS/SNP	14 (9–23)	4	4 (100, 39.8–100)	311	0 (0.00, 0.00–1.18)
Pergament (2014) <sup>71</sup>	SNP	14 (7–40)	58	58 (100, 93.8–100)	905	0 (0.00, 0.00–0.41)
Porreco (2014) <sup>72</sup>	MPSS	17 (9–37)	137	137 (100, 97.3–100)	3185	3 (0.09, 0.02–0.28)
Shaw (2014) <sup>73</sup>	MPSS	> 12	11	11 (100, 71.5–100)	184	0 (0.00, 0.00–1.98)
Stumm (2014) <sup>74</sup>	MPSS	15 (11–32)	41	40 (97.6, 87.2–99.9)	430	0 (0.00, 0.00–0.85)
Quezada (2015) <sup>75</sup>	CSS	10 (10–11)	32	32 (100, 89.1–100)	2753	1 (0.04, 0.00–0.20)
Song (2015) <sup>76</sup>	MPSS	9 (8–12)	2	2 (100, 15.8–100)	201	0 (0.00, 0.00–1.82)
Pooled analysis (% (95% CI))						
Fixed effects model				99.2 (98.5–99.6)		0.09 (0.05–0.13)
Random effects model				99.2 (98.5–99.6)		0.09 (0.05–0.14)
Cochran's Q				10.7230 ( $P=0.9858$ )		27.2044 ( $P=0.2474$ )
$I^2$ statistic (% (95% CI))				0.0 (0.0–39.6)		15.5 (0.0–48.6)
Egger bias				–0.0512 ( $P=0.6525$ )		0.2367 ( $P=0.2270$ )

Only the first author of each study is given. CSS, chromosome-specific sequencing; GA, gestational age; MPSS, massively parallel shotgun sequencing; SNP, single nucleotide polymorphism-based method.

**Table 3** Studies reporting on the application of cell-free DNA analysis of maternal blood in screening for trisomy 18 in singleton pregnancy

Study	Method	GA (weeks)	Trisomy 18		Non-trisomy 18	
			Total (n)	Detection (n (%), 95% CI)	Total (n)	False positive (n (%), 95% CI)
Chen (2011) <sup>2</sup>	MPSS	—	37	34 (91.9, 78.1–98.3)	252	5 (1.98, 0.65–4.57)
Sehnert (2011) <sup>44</sup>	MPSS	15 (10–28)	8	8 (100, 63.1–100)	39	0 (0.00, 0.00–9.03)
Ashoor (2012) <sup>45</sup>	CSS	12 (11–13)	50	49 (98.0, 89.4–99.9)	347	0 (0.00, 0.00–1.06)
Bianchi (2012) <sup>46</sup>	MPSS	15 (10–23)	36	35 (97.2, 85.5–99.9)	460	0 (0.00, 0.00–0.80)
Jiang (2012) <sup>48</sup>	MPSS	— (10–34)	12	12 (100, 73.5–100)	891	1 (0.11, 0.00–0.62)
Lau (2012) <sup>49</sup>	MPSS	12 (11–28)	10	10 (100, 69.2–100)	98	0 (0.00, 0.00–3.69)
Nicolaides (2012) <sup>50</sup>	CSS	12 (11–13)	2	2 (100, 15.8–100)	1947	2 (0.10, 0.01–0.37)
Norton (2012) <sup>51</sup>	CSS	16 (10–38)	38	37 (97.4, 86.2–99.9)	2888	2 (0.07, 0.01–0.25)
Palomaki (2012) <sup>52</sup>	MPSS	14 (9–22)	59	59 (100, 93.9–100)	1912	5 (0.26, 0.09–0.61)
Sparks (2012) <sup>53</sup>	CSS	18 (11–36)	8	8 (100, 63.1–100)	159	0 (0.00, 0.00–2.29)
Guex (2013) <sup>55</sup>	MPSS	12 (11–13)	20	19 (95.0, 75.1–99.9)	156	0 (0.00, 0.00–2.34)
Liang (2013) <sup>57</sup>	MPSS	21 (11–39)	13	13 (100, 75.3–100)	393	0 (0.00, 0.00–0.93)
Nicolaides (2013) <sup>59</sup>	SNP	13 (11–13)	3	3 (100, 29.2–100)	226	0 (0.00, 0.00–1.62)
Song (2013) <sup>61</sup>	MPSS	16 (11–21)	2	2 (100, 15.8–100)	1739	1 (0.06, 0.00–0.32)
Bianchi (2013) <sup>63</sup>	MPSS	17 (8–39)	2	2 (100, 15.8–100)	1950	3 (0.15, 0.03–0.45)
Pergament (2014) <sup>71</sup>	SNP	14 (7–40)	24	24 (100, 85.8–100)	938	0 (0.00, 0.00–0.39)
Porreco (2014) <sup>72</sup>	MPSS	17 (9–37)	39	36 (92.3, 79.1–98.4)	3283	0 (0.00, 0.00–0.11)
Shaw (2014) <sup>73</sup>	MPSS	> 12	7	7 (100, 59.0–100)	188	0 (0.00, 0.00–1.94)
Stumm (2014) <sup>74</sup>	MPSS	15 (11–32)	8	8 (100, 63.1–100)	463	1 (0.22, 0.01–1.20)
Quezada (2015) <sup>75</sup>	CSS	10 (10–11)	10	9 (90.0, 55.5–99.8)	2775	5 (0.18, 0.06–0.42)
Song (2015) <sup>76</sup>	MPSS	9 (8–12)	1	1 (100, 2.50–100)	202	0 (0.00, 0.00–1.81)
Pooled analysis (% (95% CI))						
Fixed effects model				96.3 (94.3–97.9)		0.12 (0.08–0.17)
Random effects model				96.3 (94.3–97.9)		0.13 (0.07–0.20)
Cochran's Q				11.9512 ( <i>P</i> = 0.9177)		29.7620 ( <i>P</i> = 0.0738)
<i>I</i> <sup>2</sup> statistic (% (95% CI))				0.0 (0.0–41.5)		2.8 (0–59.5)
Egger bias				–0.2031 ( <i>P</i> = 0.2831)		0.4687 ( <i>P</i> = 0.0513)

Only the first author of each study is given. CSS, chromosome-specific sequencing; GA, gestational age; MPSS, massively parallel shotgun sequencing; SNP, single nucleotide polymorphism-based method.

FPR were 91.0% (95% CI, 85.0–95.6%) and 0.13% (95% CI, 0.05–0.26%), respectively.

### Monosomy X

A total of 16 studies reported on the detection of monosomy X by cfDNA analysis, for a combined total of 177 singleton pregnancies with fetal monosomy X and 9079 with no monosomy X (Table 5). In individual studies, the DR varied between 66.7% and 100% and the FPR varied between 0% and 0.52%. The pooled weighted DR and FPR were 90.3% (95% CI, 85.7–94.2%) and 0.23% (95% CI, 0.14–0.34%), respectively.

### Sex chromosome aneuploidies other than monosomy X

A total of 12 studies reported on the performance of screening by cfDNA analysis for sex chromosome abnormalities other than monosomy X, in a combined total of 56 affected and 6699 non-sex chromosome aneuploidy singleton pregnancies (Table 6). The pooled weighted DR and FPR were 93.0% (95% CI, 85.8–97.8%) and 0.14% (95% CI, 0.06–0.24%), respectively.

### Studies in twin pregnancies

Five studies reported on the performance of screening by cfDNA analysis for trisomies in twin pregnancies

(Table 7). In a combined total of 31 trisomy-21 and 399 euploid pregnancies, the DR was 93.7% (95% CI, 83.6–99.2%) and the FPR was 0.23% (95% CI, 0.00–0.92%). There were also nine trisomy-18 pregnancies and two trisomy-13 pregnancies and these were all classified correctly<sup>47,65,69</sup>.

### Comparison with traditional methods of screening in routine populations

Four studies compared the performance of screening for trisomies by cfDNA testing with that of traditional methods of screening<sup>50,61,63,75</sup>. The first study<sup>50</sup> examined stored plasma samples from singleton pregnancies that underwent combined screening at 11–13 weeks' gestation. In the 1949 cases with both cfDNA and combined test results, all 10 trisomic pregnancies were detected by both tests, with a FPR of 0.1% for the cfDNA test and 4.5% for the combined test.

In the second study<sup>61</sup>, cfDNA testing and second-trimester triple serum screening were performed prospectively at a median gestational age of 16 (range, 11–21) weeks. In the 1741 pregnancies with cfDNA results and outcome data, the test correctly identified all 11 trisomic pregnancies, with a FPR of 0.06%; the triple test identified only 6 (54.5%) of the trisomies, with a FPR of 14.1%.

**Table 4** Studies reporting on the application of cell-free DNA analysis of maternal blood in screening for trisomy 13 in singleton pregnancy

Study	Method	GA (weeks)	Trisomy 13		Non-trisomy 13	
			Total (n)	Detection (n (%), 95% CI)	Total (n)	False positive (n (%), 95% CI)
Chen (2011) <sup>2</sup>	MPSS	—	25	25 (100, 86.3–100)	264	3 (1.14, 0.24–3.29)
Bianchi (2012) <sup>46</sup>	MPSS	15 (10–23)	14	11 (78.6, 49.2–95.3)	485	0 (0.00, 0.00–0.76)
Jiang (2012) <sup>48</sup>	MPSS	— (10–34)	2	2 (100, 15.8–100)	901	0 (0.00, 0.00–0.41)
Lau (2012) <sup>49</sup>	MPSS	12 (11–28)	2	2 (100, 15.8–100)	106	0 (0.00, 0.00–3.42)
Palomaki (2012) <sup>52</sup>	MPSS	14 (9–22)	12	11 (91.7, 61.5–99.8)	1959	16 (0.82, 0.47–1.32)
Ashoor (2013) <sup>54</sup>	CSS	13 (11–26)	10	8 (80.0, 44.4–97.5)	1949	1 (0.05, 0.00–0.29)
Guex (2013) <sup>55</sup>	MPSS	12 (11–13)	13	13 (100, 75.3–100)	163	0 (0.00, 0.00–2.24)
Liang (2013) <sup>57</sup>	MPSS	21 (11–39)	3	3 (100, 29.2–100)	403	1 (0.25, 0.01–1.38)
Nicolaides (2013) <sup>59</sup>	SNP	13 (11–13)	1	1 (100, 2.5–100)	228	0 (0.00, 0.00–1.61)
Song (2013) <sup>61</sup>	MPSS	16 (11–21)	1	1 (100, 2.5–100)	1740	0 (0.00, 0.00–0.21)
Bianchi (2013) <sup>63</sup>	MPSS	17 (8–39)	1	1 (100, 2.5–100)	1913	3 (0.16, 0.03–0.46)
Hall (2014) <sup>67*</sup>	SNP	16 (12–22)	14	14 (100, 76.8–100)	49	0 (0.00, 0.00–7.25)
Pergament (2014) <sup>71</sup>	SNP	14 (7–40)	11	11 (100, 71.5–100)	953	0 (0.00, 0.00–0.39)
Porreco (2014) <sup>72</sup>	MPSS	17 (9–37)	16	14 (87.5, 61.7–98.5)	3306	0 (0.00, 0.00–0.11)
Shaw (2014) <sup>73</sup>	MPSS	> 12	3	3 (100, 29.2–100)	192	0 (0.00, 0.00–1.90)
Stumm (2014) <sup>74</sup>	MPSS	15 (11–32)	5	5 (100, 47.8–100)	466	0 (0.00, 0.00–0.79)
Quezada (2015) <sup>75</sup>	CSS	10 (10–11)	5	2 (40.0, 52.8–85.3)	2780	2 (0.07, 0.01–0.26)
Song (2015) <sup>76</sup>	MPSS	9 (8–12)	1	1 (100, 2.5–100)	202	0 (0.00, 0.00–1.81)
Pooled analysis (% (95% CI))						
Fixed effects model				91.7 (86.9–95.5)		0.11 (0.06–0.16)
Random effects model				91.0 (85.0–95.6)		0.13 (0.05–0.26)
Cochran's Q				21.6858 ( $P = 0.1971$ )		50.2813 ( $P < 0.0001$ )
$I^2$ statistic (% (95% CI))				21.6 (0.0–55.3)		66.2 (38.7–78.2)
Egger bias				-0.6143 ( $P = 0.1104$ )		0.5732 ( $P = 0.0907$ )

Only the first author of each study is given. \*Hall reports 15 cases but one case is from Nicolaides 2013. CSS, chromosome-specific sequencing; GA, gestational age; MPSS, massively parallel shotgun sequencing; SNP, single nucleotide polymorphism-based method.

In the third study<sup>63</sup>, prospective screening by cfDNA testing at 17 (range, 8–39) gestational weeks and a variety of traditional tests (first-trimester combined test in 39%, second-trimester serum quadruple test in 23% and combinations of the first- and second-trimester tests in 38%) were performed. In the 1914 pregnancies with outcome data, both tests correctly identified all eight trisomic pregnancies, with a FPR of 0.5% for the cfDNA test and 4.2% for the traditional tests.

In the fourth study<sup>75</sup>, prospective screening by cfDNA testing was performed at 10–11 weeks' gestation and by the combined test at 11–13 weeks. In the 2785 pregnancies with cfDNA results and outcome data, the test correctly identified all 32 cases with trisomy 21, nine of 10 with trisomy 18 and two of five with trisomy 13, with a total FPR of 0.3%. The combined test correctly identified all trisomic pregnancies, with a FPR of 4.4%.

## DISCUSSION

### Performance of screening for aneuploidies

#### Screening for trisomy 21

In singleton pregnancies, cfDNA analysis of maternal blood can detect more than 99% of cases of fetal trisomy 21 with a FPR of less than 0.1%. The combined total number of affected ( $n = 1051$ ) and unaffected ( $n = 21\,608$ )

pregnancies was large and the heterogeneity between studies was low.

Although most studies were in high-risk pregnancies, there were five studies with a combined total of 57 affected and 8685 unaffected pregnancies in general populations<sup>50,61,63,64,75</sup>, with a DR of 100% and a FPR of 0.08%. In two of the latter studies<sup>50,75</sup>, the cfDNA test was compared with the first-trimester combined test in a combined total of 40 trisomy-21 and 4694 unaffected pregnancies, with DRs of 100% for both tests but a FPR of 0.02% for the cfDNA test and 4.4% for the combined test. In another study<sup>61</sup>, at a median gestational age of 16 weeks, the cfDNA test detected all cases of trisomy 21, 18 or 13, with a FPR of 0.06%, whereas the second-trimester serum triple test detected only 55% of the trisomies, with a FPR of 14.1%. In a fourth study<sup>63</sup>, at 8–39 weeks, both the cfDNA test and a range of first- and/or second-trimester traditional tests detected all cases of trisomy 21, with a FPR of 0.3% for the cfDNA test and 3.6% for the traditional tests.

#### Screening for trisomies 18 and 13

The performance of cfDNA analysis of maternal blood in the identification of singleton pregnancies with fetal trisomy 18 or 13, with respective DRs of about 96% and 91% and a combined FPR of 0.26%, is worse than is the performance of screening for trisomy 21. The objective of trying to identify all three trisomies, rather than trisomy



**Table 5** Studies reporting on the application of cell-free DNA analysis of maternal blood in screening for monosomy X in singleton pregnancy

Study	Method	GA (weeks)	Monosomy X		Non-monosomy X	
			Total (n)	Detection (n (%), 95% CI)	Total (n)	False positive (n (%), 95% CI)
Sehnert (2011) <sup>44</sup>	MPSS	15 (10–28)	2	2 (100, 15.8–100)	45	0 (0.00, 0.00–7.87)
Bianchi (2012) <sup>46</sup>	MPSS	15 (10–23)	20	15 (75.0, 50.9–91.3)	462	1 (0.22, 0.01–1.20)
Jiang (2012) <sup>48</sup>	MPSS	— (10–34)	3	3 (100, 29.2–100)	899	1 (0.11, 0.00–0.62)
Lau (2012) <sup>49</sup>	MPSS	12 (11–28)	8	8 (100, 63.1–100)	100	0 (0.00, 0.00–3.62)
Guex (2013) <sup>55</sup>	MPSS	12 (11–13)	15	15 (100, 78.2–100)	161	0 (0.00, 0.00–2.27)
Liang (2013) <sup>57</sup>	MPSS	21 (11–39)	5	5 (100, 47.8–100)	401	1 (0.25, 0.01–1.38)
Mazloom (2013) <sup>58</sup>	MPSS	— (10–20)	21	17 (81.0, 58.1–94.6)	390	1 (0.26, 0.01–1.42)
Nicolaides (2013) <sup>59</sup>	SNP	13 (11–13)	2	2 (100, 15.8–100)	227	0 (0.00, 0.00–1.61)
Samango-Sprouse (2013) <sup>60</sup>	SNP	13 (9–36)	12	11 (91.7, 61.5–99.8)	175	0 (0.00, 0.00–2.09)
Song (2013) <sup>61</sup>	MPSS	16 (11–21)	3	2 (66.7, 9.4–99.2)	1737	0 (0.00, 0.00–0.21)
Comas (2014) <sup>64</sup>	CSS/SNP	14 (9–23)	0	—	315	1 (0.32, 0.01–1.76)
Hooks (2014) <sup>68</sup>	CSS	15 (10–34)	27	26 (96.3, 81.0–99.9)	387	2 (0.52, 0.06–1.85)
Nicolaides (2014) <sup>70</sup>	CSS	12 (11–13)	47	43 (91.5, 79.6–97.6)	116	0 (0.00, 0.00–3.13)
Porreco (2014) <sup>72</sup>	MPSS	17 (9–37)	9	9 (100, 66.4–100)	3269	11 (0.34, 0.17–0.60)
Shaw (2014) <sup>73</sup>	MPSS	> 12	3	3 (100, 29.2–100)	192	0 (0.00, 0.00–1.90)
Song (2015) <sup>76</sup>	MPSS	9 (8–12)	0	—	203	1 (0.49, 0.01–2.71)
Pooled analysis (% (95% CI))						
Fixed effects model				90.3 (85.8–94.1)		0.23 (0.14–0.34)
Random effects model				90.3 (85.7–94.2)		0.23 (0.14–0.34)
Cochran's Q				13.2419 ( <i>P</i> = 0.4293)		15.2823 ( <i>P</i> = 0.4313)
<i>I</i> <sup>2</sup> statistic (% (95% CI))				1.8 (0.0–48.4)		1.8 (0.0–46.4)
Egger bias				–0.2358 ( <i>P</i> = 0.6481)		0.3781 ( <i>P</i> = 0.1668)

Only the first author of each study is given. CSS, chromosome-specific sequencing; GA, gestational age; MPSS, massively parallel shotgun sequencing; SNP, single nucleotide polymorphism-based method.

**Table 6** Studies reporting on the application of cell-free DNA analysis of maternal blood in screening for sex chromosome abnormalities (SCA) other than monosomy X in singleton pregnancy

Study	Method	GA (weeks)	47,XXX; 47,XXY; 47,XYY		Non-SCA	
			Total (n)	Detection (n (%), 95% CI)	Total (n)	False positive (n (%), 95% CI)
Bianchi (2012) <sup>46</sup>	MPSS	15 (10–23)	9	8 (88.9, 51.8–99.7)	453	0 (0.00, 0.00–0.81)
Jiang (2012) <sup>48</sup>	MPSS	— (10–34)	3	3 (100, 29.2–100)	896	0 (0.00, 0.00–0.41)
Lau (2012) <sup>49</sup>	MPSS	12 (11–28)	1	1 (100, 2.5–100)	99	0 (0.00, 0.00–3.66)
Guex (2013) <sup>55</sup>	MPSS	12 (11–13)	5	5 (100, 47.8–100)	156	0 (0.00, 0.00–2.34)
Liang (2013) <sup>57</sup>	MPSS	21 (11–39)	3	3 (100, 29.2–100)	398	1 (0.25, 0.01–1.39)
Mazloom (2013) <sup>58</sup>	MPSS	— (10–20)	8	8 (100, 63.1–100)	382	0 (0.00, 0.00–0.96)
Samango-Sprouse (2013) <sup>60</sup>	SNP	13 (9–36)	3	3 (100, 29.2–100)	172	0 (0.00, 0.00–2.12)
Hooks (2014) <sup>68</sup>	CSS	15 (10–34)	7	7 (100, 59.0–100)	380	0 (0.00, 0.00–0.97)
Nicolaides (2014) <sup>70</sup>	CSS	12 (11–13)	9	9 (100, 66.4–100)	107	1 (0.94, 0.02–5.10)
Porreco (2014) <sup>72</sup>	MPSS	17 (9–37)	6	6 (100, 54.1–100)	3263	5 (0.15, 0.05–0.36)
Shaw (2014) <sup>73</sup>	MPSS	> 12	1	1 (100, 2.5–100)	191	0 (0.00, 0.00–1.91)
Song (2015) <sup>76</sup>	MPSS	9 (8–12)	1	0 (0.0, 0.0–97.5)	202	0 (0.00, 0.00–1.81)
Pooled analysis (% (95% CI))						
Fixed effects model				93.0 (85.8–97.8)		0.14 (0.06–0.24)
Random effects model				93.0 (85.8–97.8)		0.14 (0.06–0.24)
Cochran's Q				8.7823 ( <i>P</i> = 0.6420)		6.1030 ( <i>P</i> = 0.8664)
<i>I</i> <sup>2</sup> statistic (% (95% CI))				0.0 (0.0–49.8)		0.0 (0.0–49.8)
Egger bias				–1.4222 ( <i>P</i> = 0.1776)		–0.1007 ( <i>P</i> = 0.6579)

Only the first author of each study is given. All monosomy-X pregnancies have been excluded from these data. CSS, chromosome-specific sequencing; GA, gestational age; MPSS, massively parallel shotgun sequencing; SNP, single nucleotide polymorphism-based method.

21 alone, is achieved at the expense of a four-fold increase in the FPR, from 0.09% to 0.35%. Furthermore, the number of affected cases examined, 389 for trisomy 18 and 139 for trisomy 13, was considerably smaller than that for trisomy 21, and the heterogeneity in DR and FPR between studies was much higher for trisomy 13 than for the other two trisomies.

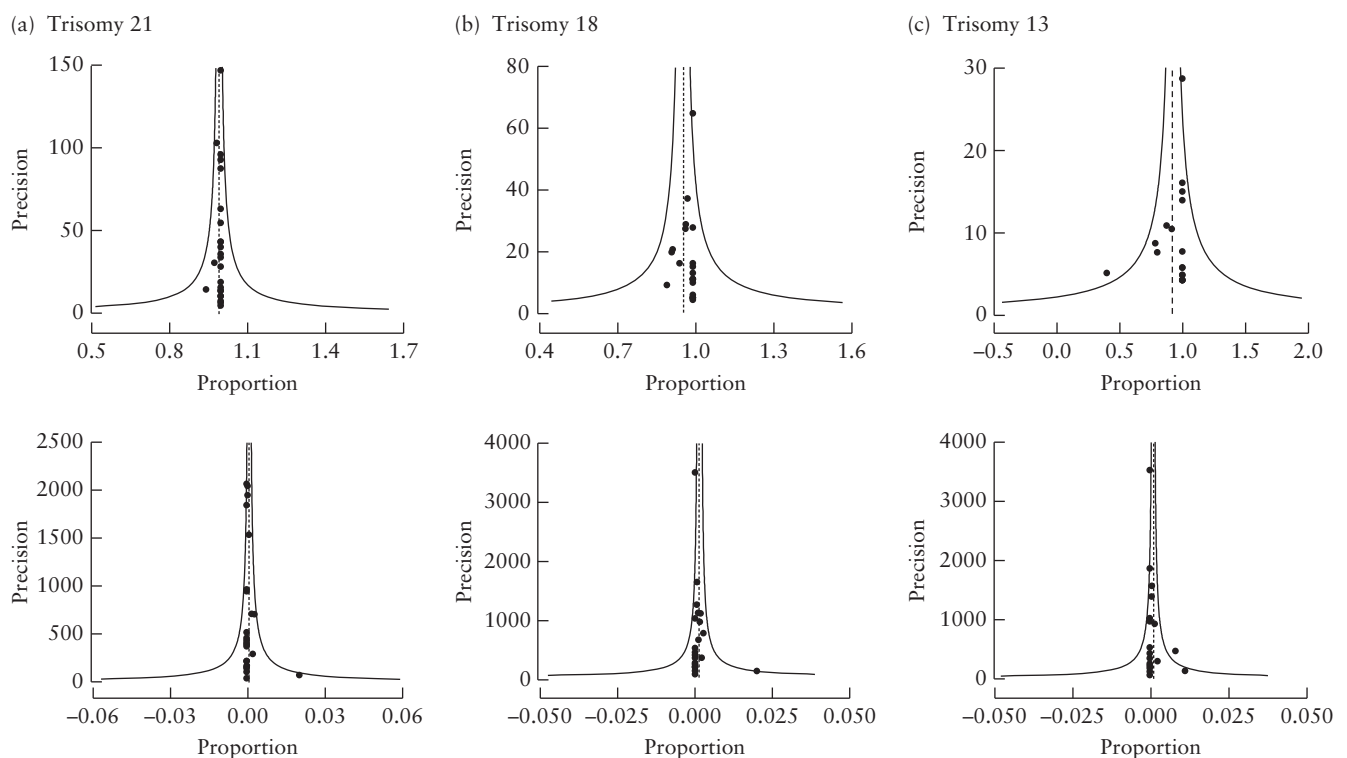
### Screening for sex chromosome aneuploidies

A small number of studies, with a combined total of 177 singleton pregnancies with fetal monosomy X and 56 with other sex chromosome aneuploidies, reported that cfDNA analysis of maternal blood detected about 90% of the former and 93% of the latter, with a combined FPR

**Table 7** Studies reporting on the application of cell-free DNA analysis of maternal blood in screening for trisomy 21 in twin pregnancy

Study	Method	GA (weeks)	Trisomy 21		Non-trisomy 21	
			Total (n)	Detection (n (%), 95% CI)	Total (n)	False positive (n (%), 95% CI)
Canick (2012) <sup>47</sup>	MPSS	14 (10–18)	7	7 (100, 59.0–100)	17	0 (0.0, 0.0–19.5)
Lau (2013) <sup>56</sup>	MPSS	13 (11–20)	1	1 (100, 2.5–100)	11	0 (0.0, 0.0–28.5)
del Mar Gil (2014) <sup>65</sup>	CSS	13 (12–13)	10	9 (90.0, 55.5–99.7)	181	0 (0.0, 0.0–2.0)
Grömminger (2014) <sup>66</sup>	MPSS	15 (10–18)	4	4 (100, 39.8–100)	12	0 (0.0, 0.0–26.5)
Huang (2014) <sup>69</sup>	MPSS	19 (11–36)	9	9 (100, 66.4–100)	178	0 (0.0, 0.0–2.1)
Pooled analysis (% (95% CI))						
Fixed effects model				93.7 (83.6–99.2)		0.23 (0.00–0.92)
Random effects model				93.7 (83.6–99.2)		0.23 (0.00–0.92)
Cochran's Q				1.3097 (P=0.8597)		1.4391 (P=0.8374)
I <sup>2</sup> statistic (% (95% CI))				0.0 (0.0–64.1)		0.0 (0.0–64.1)
Egger bias				-0.0239 (P=0.0833)		—

Only the first author of each study is given. CSS, chromosome-specific sequencing; GA, gestational age; MPSS, massively parallel shotgun sequencing.



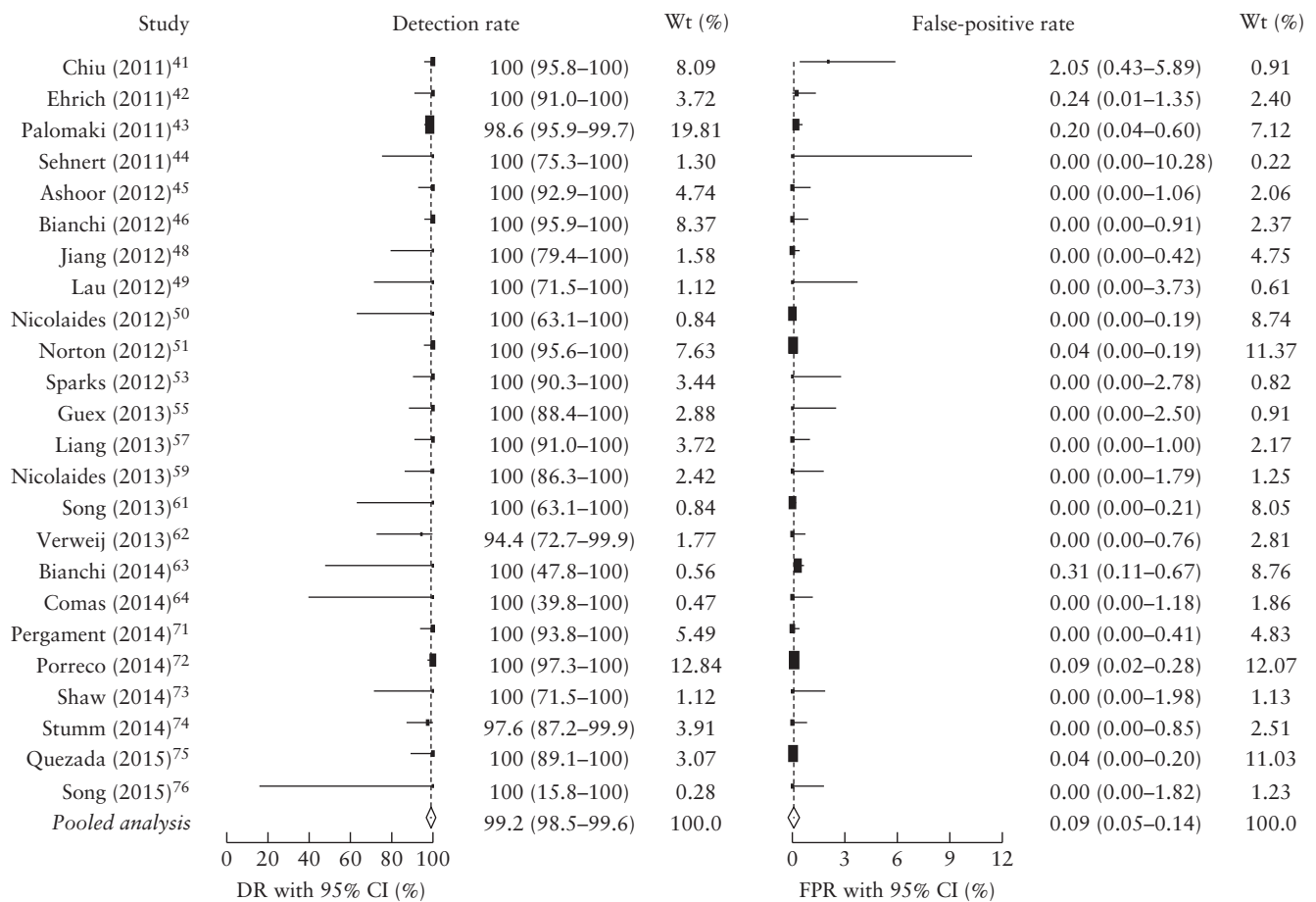
**Figure 3** Funnel plots demonstrating assessment of publication bias in screening for trisomies 21 (a), 18 (b) and 13 (c). Top panel gives results for detection rate and bottom one for false-positive rate.

of 0.37%. Certainly in some studies the rate of laboratory failure to provide a result was considerably higher for sex chromosome aneuploidies than it was for the trisomies.

#### Screening for aneuploidies in twin pregnancies

In twin pregnancies, while screening by cfDNA testing is feasible, the performance of screening may be worse than it is in singletons. In twins, cfDNA testing is more complex, because the two fetuses could be either monozygotic, and therefore genetically identical, or dizygotic, in which case only one fetus is likely to have any

aneuploidy identified. There is evidence that, in dizygotic twins, each fetus can contribute different amounts of cfDNA into the maternal circulation, and the difference can be nearly two-fold<sup>16,81</sup>. It is therefore possible, in a dizygotic twin pregnancy discordant for aneuploidy, for the fetal fraction of the affected fetus to be below the threshold (4%) for successful cfDNA testing. This could lead to an erroneous result of low risk for aneuploidy, with a high contribution from the disomic cotwin resulting in a satisfactory total fetal fraction. To avoid this potential mistake, it was proposed that for cfDNA testing in twin pregnancies, the lower fetal fraction of the two fetuses, rather than the total fetal fraction, should be estimated



**Figure 4** Forest plots of detection rates (DR) and false-positive rates (FPR) with 95% CIs and weighted pooled summary statistics using the random-effects model in assessing cell-free DNA analysis in screening for trisomy 21 in singleton pregnancy. Only the first author of each study is given.

in the assessment of risk for aneuploidies<sup>82</sup>. However, an inevitable consequence of such a policy is that the no-result rate in twins is higher than that in singleton pregnancies<sup>39</sup>.

#### Methodological quality of the studies in the meta-analysis

In the assessment of methodological quality by QUADAS-2<sup>77</sup>, most studies were considered to be at high risk of bias and at high risk of concerns regarding applicability in relation to patient selection. This is essentially because most studies were performed in selected 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<sup>45,50</sup>. This is supported by the finding that the performance of the test in the five studies that were carried out in a general population<sup>50,61,63,64,75</sup> 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 performed. The real issue in relation to the failure rate in cfDNA testing is whether this is higher in aneuploid compared with euploid fetuses. A common cause of failure of the test to provide a result is low fetal fraction. The fetal fraction increases with increasing serum pregnancy-associated plasma protein-A and free  $\beta$ -human chorionic gonadotropin and is inversely related to maternal weight; the levels are not significantly altered in pregnancies with fetal trisomy 21 but they are reduced in those with trisomy 18<sup>83,84</sup>. It is therefore expected that, in trisomies 18 and 13, the failure rate of the cfDNA test would be increased, thereby introducing bias if only the cases with results are included in the calculation of the performance of screening. One study has reported that the rate of failed results was considerably higher in aneuploid than in euploid pregnancies<sup>71</sup>.

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

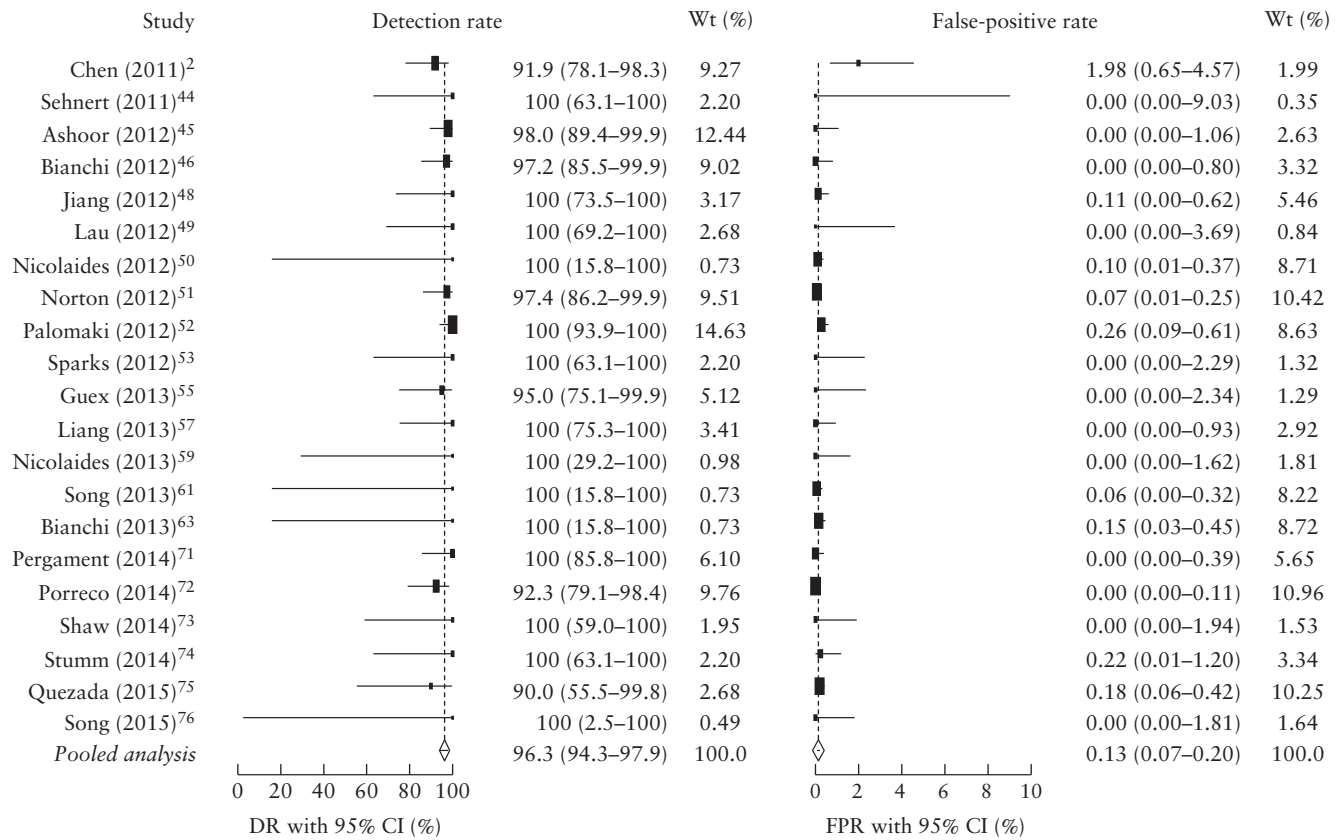


Figure 5 Forest plots of detection rates (DR) and false-positive rates (FPR) with 95% CIs and weighted pooled summary statistics using the random-effects model in assessing cell-free DNA analysis in screening for trisomy 18 in singleton pregnancy. Only the first author of each study is given.

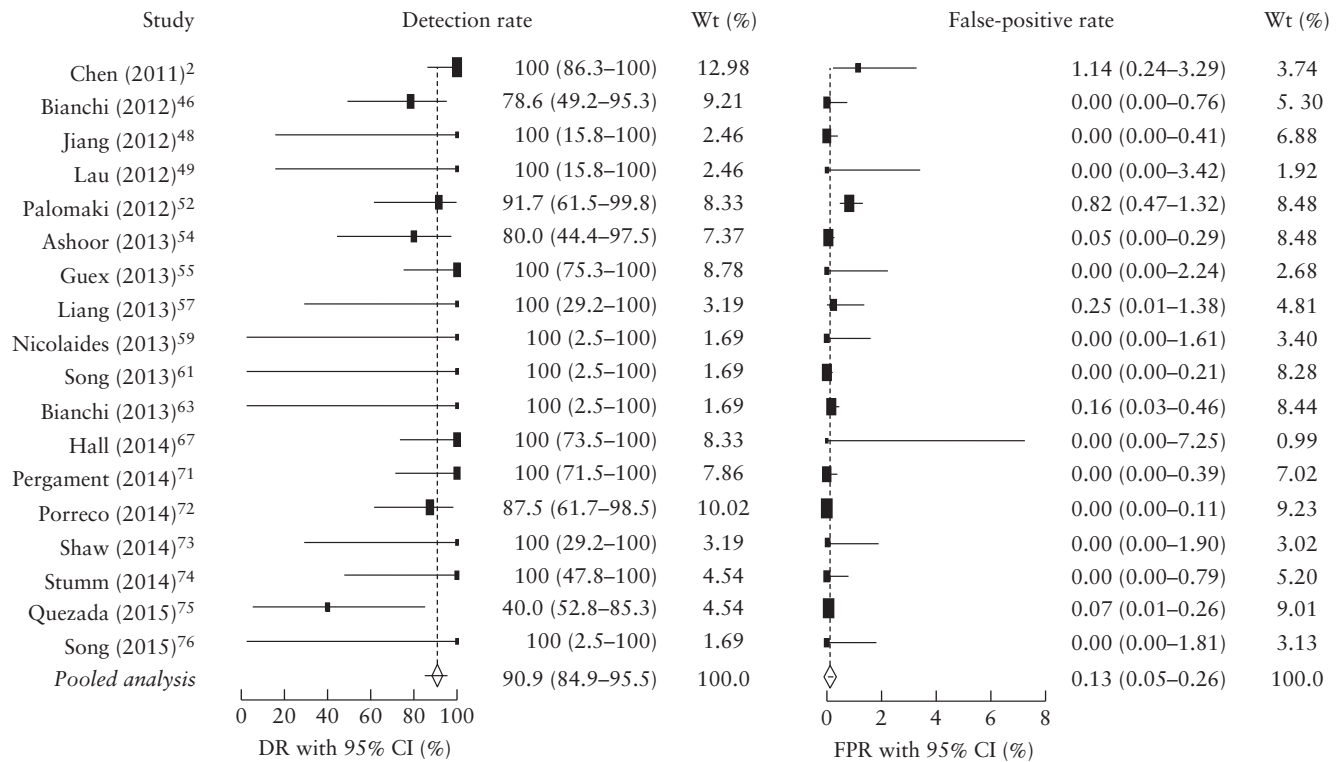
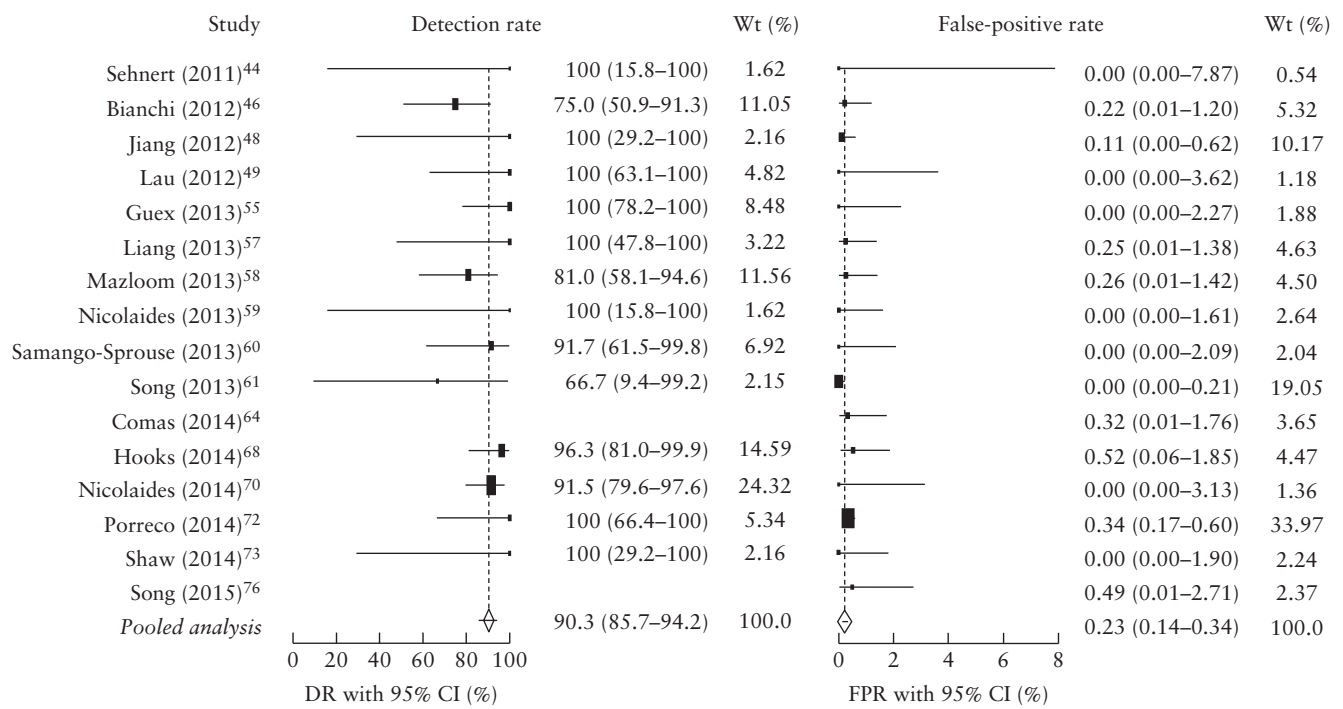
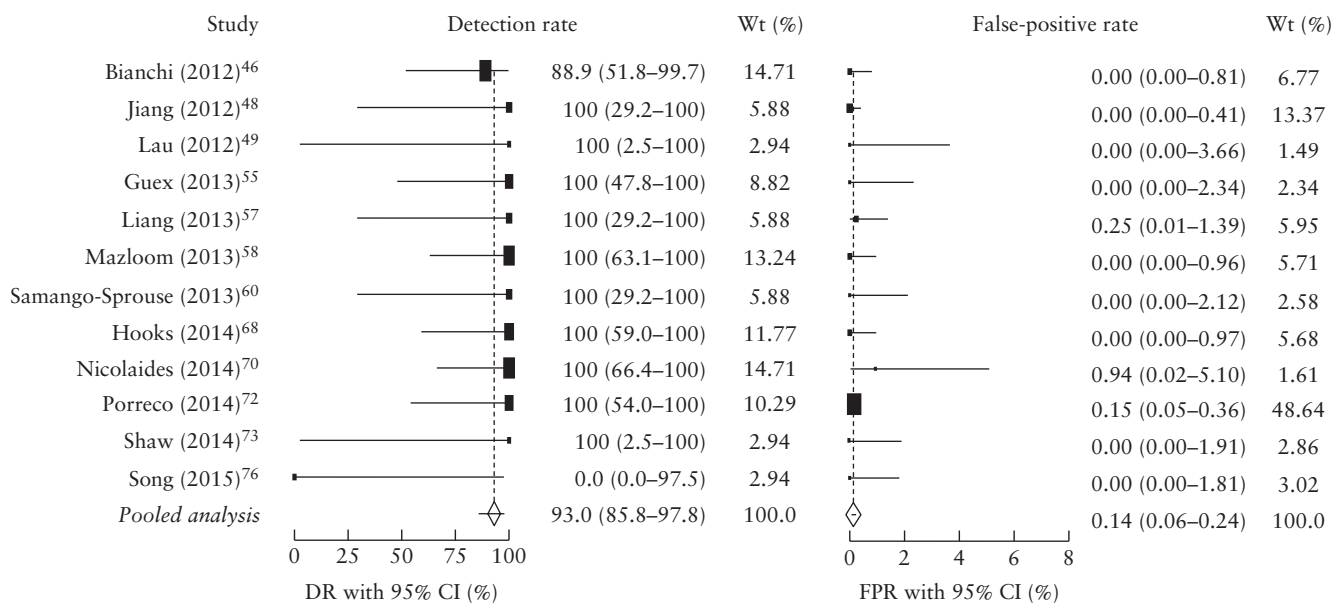


Figure 6 Forest plots of detection rates (DR) and false-positive rates (FPR) with 95% CIs and weighted pooled summary statistics using the random-effects model in assessing cell-free DNA analysis in screening for trisomy 13 in singleton pregnancy. Only the first author of each study is given.



**Figure 7** Forest plots of detection rates (DR) and false-positive rates (FPR) with 95% CIs and weighted pooled summary statistics using the random-effects model in assessing cell-free DNA (cfDNA) analysis in screening for monosomy X in singleton pregnancy. Only the first author of each study is given.



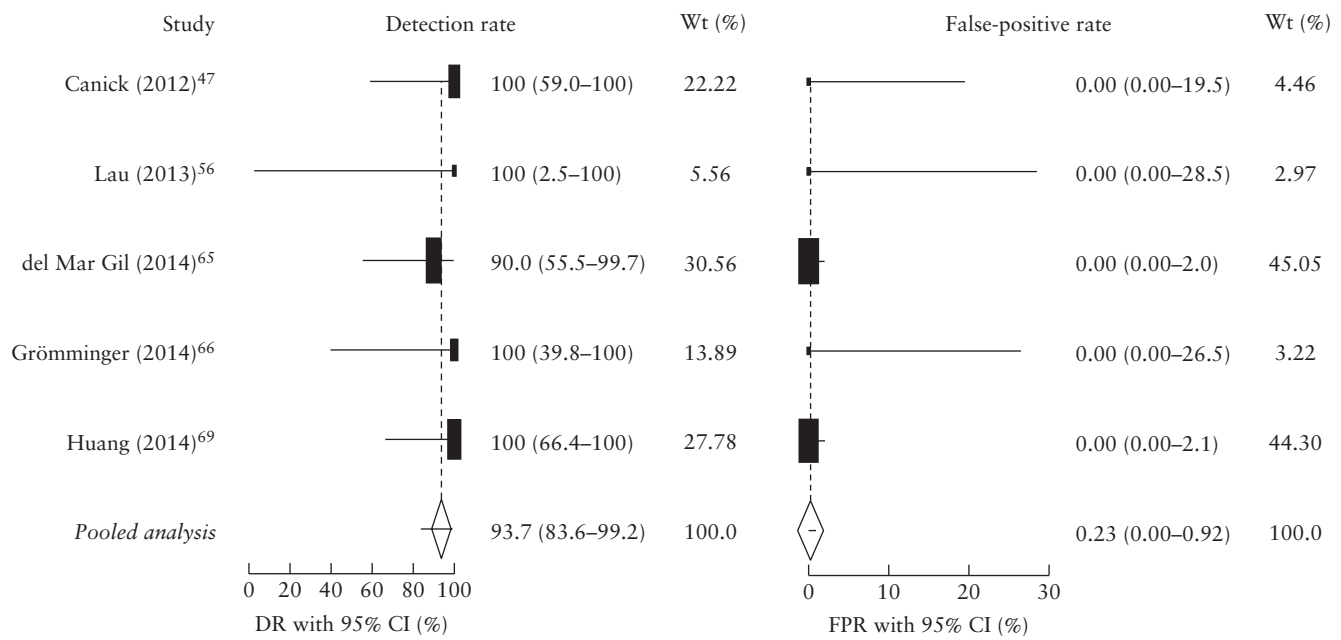
**Figure 8** Forest plots of detection rates (DR) and false-positive rates (FPR) with 95% CIs and weighted pooled summary statistics using the random-effects model in assessing cell-free DNA (cfDNA) analysis in screening for sex chromosome abnormalities other than monosomy X in singleton pregnancy. Only the first author of each study is given.

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 chromosome aneuploidies by clinical examination of the neonate is not reliable; consequently, there are real 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.

**Clinical implications**

*Trisomy 21*

There is clear evidence that in singleton pregnancies the performance of screening for trisomy 21 by cfDNA testing is superior to that of all other methods combining maternal age, first- or second-trimester ultrasound findings and first- or second-trimester serum biochemical



**Figure 9** Forest plots of detection rates (DR) and false-positive rates (FPR) with 95% CIs and weighted pooled summary statistics using the random-effects model in assessing cell-free DNA (cfDNA) analysis in screening for trisomy 21 in twin pregnancies. Only the first author of each study is given.

analysis. Additionally, the test can be carried out at 10–11 weeks' gestation, with the advantage of providing early reassurance for the majority of parents that their fetus is unlikely to be trisomic and, for the few with an affected fetus, the parents have the option of an earlier and safer termination of pregnancy<sup>31,75</sup>.

There are essentially two options in the clinical implementation of cfDNA analysis of maternal blood in screening for trisomy 21: first, routine screening of the whole population, and second, contingent screening based on the results of first-line screening by another method, preferably the first-trimester combined test. The two major limitations of cfDNA testing as a potential method for universal screening are the high cost of the test and the rate of failure to provide a result. Both of these problems can be overcome by the use of cfDNA testing contingent on the results of the first-trimester combined test<sup>1,85–87</sup>. Contingent screening would lead to a very high DR and very low invasive testing rate at a considerably lower cost than compared with carrying out cfDNA testing as a first-line method of screening. In cases of failed cfDNA test, pregnant women can rely on the results of the combined test in deciding in favor or against invasive testing. This strategy would also retain the advantages of first-trimester testing by ultrasound and biochemistry, including accurate pregnancy dating, early detection of many major fetal defects and prediction, with the potential of prevention, of a wide range of pregnancy complications, including pre-eclampsia and preterm birth<sup>88</sup>.

#### Trisomies 18 and 13

There are no advocates of screening for fetal trisomies 18 and 13 independently from screening for trisomy 21. In

traditional testing, detection of these lethal trisomies has been considered to be the mere beneficial consequence of screening for trisomy 21. Large studies utilizing the first-trimester combined test have reported that use of risk algorithms for each of the three trisomies results in DRs of about 90% for trisomy 21 and 95% for trisomies 18 and 13, with an increase in FPR of only 0.1% above the FPR of about 4% in screening for trisomy 21 alone<sup>89–91</sup>.

Data from this meta-analysis of studies on cfDNA testing suggest that the performance of screening for trisomies 18 and 13 may be worse than that of the combined test. Although the reported DR of the two tests is similar, it is likely that the true DR of the cfDNA test will be lower if the cases in which the test fails to give a result are included. Furthermore, the differential increase in FPR by including these trisomies in a screening strategy aimed primarily at detecting trisomy 21 is considerably higher with cfDNA testing than with the combined test.

#### Sex chromosome aneuploidies

Conventional prenatal screening has never sought directly to uncover fetal sex chromosome aneuploidies, and their detection was coincidental in pregnancies undergoing invasive testing following screening for trisomy 21<sup>92,93</sup>. The introduction of cfDNA analysis of maternal blood has now made it possible to screen not only for trisomies 21, 18 and 13, but also potentially for other chromosomal abnormalities, including sex chromosome aneuploidies. Cases of sex chromosome aneuploidy are generally mild, without physical or intellectual disability. The only exception is the lethal type of monosomy X which presents with a very large nuchal translucency during the first trimester or cystic hygroma/hydrups during

the second trimester; in such cases the investigation of choice would be invasive testing for fetal karyotype evaluation, including subchromosomal analysis with microarray, rather than cfDNA testing for assessment of risk for 45,X.

It may be inappropriate to offer pregnant women screening for sex chromosome aneuploidies by cfDNA testing just because it is feasible. There are several reasons for this: first, the phenotype of these aneuploidies is generally mild; second, the test has a high failure rate and relatively low DR and high FPR; third, fetal mosaicism accounts for up to 50% of these aneuploidies; and fourth, the test may uncover a previously unknown maternal aneuploidy; up to 90% of women with 47,XXX are not aware that they have a third X chromosome<sup>94–96</sup>.

## Conclusions

Traditionally, screening for fetal aneuploidies has focused on trisomy 21 and, with each new method of screening introduced over the last four decades, the two objectives have been to increase the DR and decrease the rate of unnecessary invasive tests. There is now conclusive evidence that cfDNA analysis of maternal blood in screening for trisomy 21 in singleton pregnancies is superior to all previous methods in achieving both of these objectives. Performance of screening in twins by cfDNA testing requires further evaluation.

The DR of screening by cfDNA testing for trisomies 18 and 13 and sex chromosome aneuploidies is lower than that for trisomy 21. Indeed, the reported DR for these aneuploidies in this meta-analysis is likely to have been overestimated; trisomies 18 and 13 are over-represented in the cases of a failed result and sex chromosome aneuploidies are ascertained inadequately in some of the studies. Additionally, expansion of the indications of cfDNA testing to include trisomies 18 and 13 and sex chromosome aneuploidies would increase the cumulative FPR eight-fold, from 0.09% to 0.72%.

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