Antenatal screening for fetal trisomies using microarray-based cell-free DNA testing – a systematic review and meta-analysis

Fetal trisomy screening using microarray-based cell-free DNA testing

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Conflicts of interests

The authors declare that they have no conflicts of interest.

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What is already known about the topic?

- NIPT using cfDNA analysis with next-generation sequencing has high sensitivity and specificity for fetal trisomy 21 detection, with slightly lower sensitivity for trisomy 18 and 13.

What does this study add?

- This systematic review found high sensitivity and specificity of NIPT using a cfDNA test approach with microarray quantitation for the detection of fetal trisomy 21, 18 and 13. One head-to-head study showed comparable accuracy between microarray-based and sequencing-based cfDNA testing. Studies were at high risk of bias.

53 **Ethical approval and consent to participate**

54 Not applicable (systematic review of the literature).

55

56 **Data availability statement**

57 The datasets supporting the conclusions of this article are included within the article and its additional files.

Abstract

Objective To evaluate the test accuracy of non-invasive prenatal testing (NIPT) for fetal trisomy 21, 18 and 13 using cell-free (cf) DNA analysis in maternal plasma with microarray quantitation.

Method Systematic review and meta-analysis. Searches in MEDLINE, Pre-MEDLINE, EMBASE, Web of Science, and the Cochrane Library to 09.07.2018.

Results Five studies analysing 3,074 samples, including 187 trisomy 21, 43 trisomy 18, and 19 trisomy 13 cases, were identified. Risk of bias was high in all studies, introduced particularly by exclusions from analysis and by the role of the sponsor. Sensitivity of microarray-based cfDNA testing was 99.5% (95%CI 96.3% to 99.9%) for trisomy 21, 97.7% (95%CI 87.9% to 99.6%) for trisomy 18, and 100% (95%CI 83.2% to 100%) for trisomy 13. Specificity was 100% (95% CI 99.87% to 100%) for trisomy 21, 99.97% (95%CI 99.81% to 99.99%) for trisomy 18, and 99.97% (95%CI 99.81% to 99.99%) for trisomy 13. Pooled test failure rate was 1.1%. A direct comparison of microarray- and sequencing-based cfDNA found equivalent test accuracy.

Conclusion

Included studies suggest that NIPT using microarray-based cfDNA testing has high sensitivity and specificity for detecting fetal trisomy 21, 18 and 13. However, the evidence-base is small and at high risk of bias.

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Introduction

Non-invasive prenatal testing (NIPT) using cell-free DNA (cfDNA) is a method for testing for trisomies in the fetus, using a peripheral sample of the pregnant mother's blood. Three main cfDNA testing strategies had been used for trisomy screening, which all rely on next-generation sequencing (NGS) to quantify cfDNA: random whole-genome sequencing, targeted sequencing of selected nonpolymorphic regions (digital analysis of selected regions, DANSR) or targeted sequencing of single-nucleotide polymorphisms. Systematic reviews have suggested that NGS-based cfDNA strategies have a high sensitivity and specificity for detecting trisomy 21 (T21), trisomy 18 (T18) and trisomy 13 (T13).[1-3] However, most of the identified studies were prone to bias, especially in terms of the selection of participants and study flow and timing.

Recently, a microarray-based NIPT assay has been developed for fetal trisomy screening.[4] DNA microarray is a technology in which thousands of nucleic acids are bound to a surface and are used to measure the relative concentration of target nucleic acid sequences in a mixture via hybridisation and subsequent detection of the hybridisation events.[5] DNA microarray imaging is a rapid process and might allow greater sample throughput and lower costs compared to NGS-based methods.[4] The workflow of the different approaches to cfDNA testing is presented in Figure 1. The red oval marks the single step that has changed in the DNA microarray-based cfDNA test method compared to the sequencing-based methods which were included in previous reviews. To date, data on the test accuracy of microarray-based cfDNA testing for trisomy 21, 18 and 13 have not been synthesised. This systematic review examined the scientific evidence on prenatal cfDNA screening for fetal trisomy 21, 18 and 13 in relation to test accuracy and test failures in cfDNA testing approaches using DNA microarray technology for DNA quantification.

Methods

The protocols for the previous[2,6] and current review are registered at the PROSPERO International Prospective Register of Systematic Reviews (CRD42014014947 and CRD42018110314). No ethical approval was required because data from previous published studies in which informed consent was obtained by primary investigators were analysed.

Identification and selection of studies

We updated the searches from our previous review that searched electronic databases from 01.01.1997–09.02.2015.[2,6] Searches were conducted in MEDLINE (OVID), MEDLINE In-Process & Other Non-Indexed Citations (OVID), EMBASE, Web of Science, and the Cochrane Library. The search strategy combined search terms for the cfDNA test and trisomies. Database searches were updated on 9 July 2018, and were limited to articles published since 9 February 2015 (i.e. the final search date of the previous review) that were in the English language. The search strategies are provided in Appendix 1 and Appendix 2. We also contacted experts in the field and screened references of included studies and relevant systematic reviews. We supplemented the evidence with relevant studies identified in our previous review.[2,6]

Inclusion criteria were English language journal articles which investigated NIPT using cfDNA derived from maternal blood (serum, plasma, whole blood) with microarray-based DNA quantitation as screening test for fetal trisomies 21, 18 or 13 (including mosaicism and translocation), and a reference standard of either genetic verification through amniocentesis, chorionic vilus sampling (CVS), cordocentesis, fetal pathologic examination after abortion, or postnatal newborn examination followed by detailed genetic analysis, when trisomy was suspected. We included studies with any test accuracy outcomes, rates of test failure or indeterminate results. We excluded studies reporting the quantification of fetal cells, measurement of total DNA levels in maternal blood, or using epigenetic markers as a screening tool,

studies using a cfDNA test not based on DNA microarrays, studies with unclear cfDNA test technology (e.g. use of several commercially available tests), and studies using not solely a cfDNA test with DNA microarray approach for DNA quantitation and no separate data for the subgroup analysed with the microarray-based test available. We excluded case-control studies with fewer than 15 trisomy cases and cohort studies with fewer than 50 pregnant women, non-English studies, letters, reviews, editorials, grey literature, conference abstracts and communications containing insufficient information on methods and no numerical outcomes data.

Two reviewers independently screened the titles/abstracts of records identified by the update searches. Disagreements were resolved by consensus or retrieval of the full publication. Full copies of all studies deemed potentially relevant were obtained and two reviewers independently assessed these for inclusion. Disagreements were resolved by consensus or discussion with a third reviewer. Records rejected at full text stage and reasons for exclusion were documented (see Appendix 3).
The reviewers contacted the corresponding authors of potentially relevant articles if it was unclear from the publication if DNA microarrays were used for DNA quantitation for all analysed samples and/or for provision of subgroup data (see Appendix 4). As some of the corresponding authors did not know whether a microarray-based cfDNA test methodology was used, the reviewers were directed by authors to contact the laboratory that performed the cfDNA test (Ariosa Diagnostics Inc., San Jose, CA). The laboratory confirmed that they started running their samples on microarray on 10th November 2014; we therefore excluded studies with samples analysed by the concerned laboratory prior to November 2014. In some cases, there was disagreement between the laboratory and the authors about which testing technology was used, in these cases we assumed that the laboratory was correct. All correspondence was via email and is available on request.

Data were extracted by one reviewer, using a piloted data extraction form, and checked by a second reviewer. Disagreements were resolved by consensus or discussion with a third reviewer.

Quality appraisal

Quality appraisal of diagnostic accuracy studies was conducted using the same tailored QUADAS-2 tool[7] from our previous review.[2,6] This included three modifications; we also added an additional domain on the role of the sponsor to the QUADAS-2 tool (see Appendix 6). Guidance notes for the QUADAS-2 signalling questions are provided in Appendix 7. Quality assessment was undertaken by one reviewer and checked by a second reviewer. Disagreements were resolved by iteration, discussion and consensus. If required, we consulted a third reviewer.

Data synthesis

We extracted data on true and false positives, and true and false negatives from the primary studies to calculate the test accuracy measures sensitivity and specificity.

We meta-analysed studies of test accuracy using the metandi and xtmelogit functions in the Stata software package (version 15.0; Statacorp, College Station, Texas, USA). We excluded studies with no cases of trisomies 21, 18 or 13 from the bivariate meta-analysis. Where there was no heterogeneity in either sensitivity or specificity, meta-analysis cannot calculate CIs. In this case, we followed the methods of the 2017 Cochrane review[3] to sum all of the studies and calculate CIs using the Wilson method.[8,9]

To test whether the DNA microarray-based version of a cfDNA test can be considered equivalent to the NGS-based version, head-to-head test accuracy studies comparing both versions in the same cohort with a reference standard is the most informative study design. We planned to meta-analyse these studies with test type as a covariate in a bivariate model. However, as there was only one study of this type,[4] we compared sensitivity and specificity of the two test versions using McNemar's test.

We also applied the estimates of sensitivity, specificity and test failure rate of microarray-based cfDNA testing that we obtained to a hypothetical cohort of 10,000 pregnant women deemed at higher chance of fetal trisomies. We defined population prevalence of the three trisomies of interest as the median prevalence for the studies enrolling higher-chance groups as determined in the previous review (3.33% for trisomy 21, 1.5% for trisomy 18 and 0.5% for trisomy 13).[2,6]

Results

Study selection

The update searches yielded 1,891 unique results. Four records were judged to be relevant. One additional article[4] was identified from our previous searches (date limit 01/1997 to 02/2015)[2,6] resulting in a total of five included studies. Figure 2 shows the flow of records through the review. Details of excluded records are provided in Appendix 3 and Appendix 5.

Characteristics of included studies

Characteristics of the individual studies are provided in Table 1 and Appendix 8.

Study design and populations

Of the five included studies, one used a randomised controlled trial design,[10] three were cohort studies,[4,11,12] and one used an uncontrolled before-after design.[13] Two studies were single centre studies from Germany[10] and Spain,[13] one study included three centres in Canada,[11] and two studies were performed in-house by the manufacturer of the cfDNA test and retrospectively analysed frozen samples from an unclear number of centres.[4,12]

The number of women included ranged from 799[12] to 6,011[13] per study. Not all women were offered cfDNA testing; the number ranged from 72[13] to 1,198.[11] In total, 3,074 samples were successfully tested

using a microarray-based cfDNA test and had a suitable reference standard, including 187 T21, 43 T18, and 19 T13 cases. Four studies included women with singleton pregnancies only,[4,10,11,13] and one study included a mixed population of singleton and twin pregnancies (759 and 40 women, respectively).[12] Two studies compared the accuracy of cfDNA testing and standard screening approaches in first trimester pregnant women with low (no fetal ultrasound anomalies detected)[10] or general (no prior testing)[11] chance of fetal trisomies. One study reported the test accuracy of cfDNA testing in first trimester pregnant women at higher chance of fetal trisomies (first trimester combined screening showed chance >1:250 without fetal anomalies detected on ultrasound).[13] The two remaining studies assessed the test accuracy of microarray-based cfDNA testing in populations with high trisomy prevalence and an unclear proportion of first trimester pregnancies.[4,12] One of these studies also performed a head-to-head comparison of DNA microarray versus NGS as DANSR assay product quantitation methods.[4]

Testing strategies

All five studies used the Harmony Prenatal Test (Ariosa Diagnostics, Inc., San Jose, CA) which is based on the Digital Analysis of Selected Regions (DANSR) targeted approach and amplifies DNA targets from non-polymorphic regions on chromosomes 21, 18 and 13. DANSR assay products were quantified using custom DNA microarrays for all study samples. Analyses were performed by Ariosa Diagnostics Inc. (San Jose, CA) in four studies[4,11-13] and by Cenata GmbH (Tuebingen, Germany) in one study.[10] The Fetal Fraction Optimized Risk of Trisomy Evaluation (FORTE) was used to estimate the chance of having a baby with trisomy 21, 18 or 13. A higher-chance result was defined as a chance ≥1:100 in four studies,[4,10,12,13] while the cut-off used was not reported in one study.[11]

Quality appraisal

Risk of bias and applicability concerns in the five included studies are summarised in Appendix 9 (A-C).

Risk of bias was high in two or more domains in 3/5 studies[4,12,13] and high in one domain in 2/5 studies.[10,11] No study was judged to be at low risk of bias in all five domains. The study flow (exclusions from analysis) presented the area with the greatest risk of bias as all studies excluded women from analysis due to test failures and/or non-available results of newborn examination or genetic testing (pre- or postnatal). Another issue was the role of sponsor with only 1/5 studies stating that the role of sponsor played no part in design, conduction and publication.[11]

Applicability concerns were judged separately for the intended use of cfDNA testing. For cfDNA testing replacing the current primary screening test, cfDNA testing should have been performed in first-trimester pregnant women without prior testing in at least 80% of women (general obstetric population). For cfDNA testing as follow-on test in first-trimester pregnant women deemed at higher chance following a primary screening test, cfDNA testing should have been performed in first-trimester pregnant women with prior first-trimester combined test in at least 80% of women. There were significant concerns regarding the applicability of the included patient spectrum to cfDNA testing introduction in the first trimester in 3/5 studies as one study included less than 80% first-trimester pregnant women,[12] two studies performed in-house by the manufacturer were (apparently) enriched for fetal trisomies,[4,12] and in one study evaluating cfDNA testing as a replacement test, a prior ultrasound was performed and cfDNA testing was not offered to pregnant women with fetal ultrasound anomalies detected.[10] Applicability concerns regarding the index test, its conduct or interpretation were low in all five studies.

Overall test accuracy

Forest plots of the sensitivity and specificity of microarray-based cfDNA testing for all three trisomies are given in Figure 3. Test accuracy estimates from individual studies are reported in Appendix 10.

Trisomy 21 (T21)

For trisomy 21, there were a total of 186 true positives, 2,887 true negatives, 0 false positives and 1 false negative in the five included studies. One study[10] was excluded from the bivariate meta-analysis as there were no cases of T21. Pooled sensitivity was 99.5% (95%CI 96.3% to 99.9%) and pooled specificity was 100% (95%CI 99.87% to 100%).

Trisomies 18 and 13 (T18 and T13)

Bivariate meta-analysis was not possible for trisomies 18 or 13 because 3/5 studies contained no cases of these two trisomies. For trisomy 18, there were 42 true positives, 3,030 true negatives, 1 false positive and 1 false negative in the five included studies. Summing across all five studies gave a sensitivity of 97.7% (95%CI 87.9% to 99.6%), and a specificity of 99.97% (95%CI 99.81% to 99.99%). For trisomy 13, there were 19 true positives, 3,054 true negatives, 1 false positive and 0 false negatives. Summing across all five studies gave a sensitivity of 100% (95%CI 83.2% to 100%), and a specificity of 99.97% (95%CI 99.81% to 99.99%).

Direct comparison of DNA microarray-based versus NGS-based cfDNA testing

Only one head-to-head test accuracy study was identified.[4] This therefore precluded conducting the planned meta-analysis for this type of study. The included study was rated as at high risk of bias in three QUADAS-2 domains ('patient selection', 'flow & timing' and the added 'role of sponsor' domain) as women were not enrolled consecutively or randomly, the study population was enriched for fetal trisomies and it was unclear how the samples were chosen, test failures were excluded from the study, only a subset of 392 out of 878 women had an acceptable reference standard according to our inclusion criteria and could be included in our analysis, and the study was designed and conducted in-house by the manufacturer. Unpublished data for this subpopulation were provided by Ariosa Diagnostics Inc. (San Jose, CA). This study was also judged as having high applicability concerns regarding the patient spectrum as the trisomy prevalence was higher than expected even for a higher-chance population

(187/878 [1:4.7]; 18 T13, 37 T18, 132 T21, 691 normal). For both DNA microarray and sequencing technologies, this study had 72, 13 and 7 true positives for T21, T18 and T13, respectively, with the remainder being true negatives. Sensitivity and specificity estimates for both tests were 100% for all three trisomies. The difference in sensitivity between the two tests was 0% (95%CI -1.4% to +1.4%) for T21, 0% (95%CI -7.7% to +7.7%) for T18, and 0% (95%CI -14.3% to +14.3%) for T13. The difference in specificity between the two tests was 0% (95%CI -0.3% to 0.3%) for T21, 0% (95%CI -0.3% to 0.3%) for T18, and 0% (95%CI -0.3% to 0.3%) for T13.

Test failures

The rate of initial analytic failure ranged from 0.9% to 1.9% in four studies (see Appendix 11 for details).[10-13] Repeat tests after a second blood sample were successful in 1/1 women[13] and 5/11 (45.5%) women.[11] The main reason for cfDNA test failure was insufficient circulating fetal DNA in 8/8[12] and 10/11[11] samples. The only paper directly comparing DNA microarray-based versus NGS-based cfDNA testing using the DANSR approach included only samples in the study that met quality control thresholds for both quantitation methods.[4] The number of samples that failed quality control was not reported. Summing across the four studies which reported test failures gives 30 initial test failures per 2,706 samples (1.1%; 95%CI 0.8% to 1.6%).

Interpreting meta-analysis results in a higher-chance population

We applied the estimates of sensitivity, specificity and test failure rate of microarray-based cfDNA testing which we had obtained to a hypothetical cohort of 10,000 pregnant women deemed at higher chance of fetal trisomies (Figure 4). When all 10,000 pregnant women with a chance of ≥1:150 after the first trimester combined test are undergoing microarray-based cfDNA testing as follow-on test, there will be an estimated 111 women with an initial test failure (95%CI 76 to 160). In the remaining 9,889 pregnancies with successful cfDNA test, 328 (95%CI 317 to 329) cases of trisomy 21 could be detected and two cases (95%CI 0 to 12) could be missed by cfDNA testing. For trisomy 18, 145 (95%CI 130 to 148)

cases could be detected and three cases (95%CI 1 to 18) cases could be missed by cfDNA testing. For trisomy 13, 49 (95%CI 41 to 49) cases could be detected and none (95%CI 0 to 8) be missed by cfDNA testing. Of the 9,362 pregnancies not affected by one of the three trisomies, none (95%CI 0 to 12) would receive a false positive result for trisomy 21, three (95%CI 1 to 19) women would receive a false positive result for trisomy 18, and three (95%CI 1 to 19) women would receive a false positive result for trisomy 13.

Discussion

In this review, we evaluated the test accuracy of NIPT for fetal trisomy 21, 18 and 13 using cfDNA analysis in maternal plasma with microarray quantitation. Five studies met our inclusion criteria. In total, 3,074 samples were successfully analysed and had a suitable reference standard, including 187 T21, 43 T18, and 19 T13 cases. The QUADAS-2 results indicate a high risk of bias in the published evidence, introduced particularly by exclusions from analysis and by the role of the sponsor. There were also significant concerns regarding applicability of the included patient spectrum to cfDNA testing introduction in the first trimester in 3/5 studies.

This review found very high sensitivities and specificities of microarray-based cfDNA testing for the detection of fetal trisomies 21, 18 and 13. However, the evidence base identified was limited in terms of number of studies, participants and trisomy cases, resulting in considerable statistical uncertainty for the detection of trisomies 18 and 13. For comparison, our meta-analysis published in 2016[2] included 38, 33 and 25 studies using NGS-based approaches for fetal T21, T18, and T13 detection, respectively, with over 168,500 maternal samples successfully tested, of which 2,214 were affected by trisomy 21, 665 by trisomy 18 and 165 by trisomy 13. No evidence of a difference (rather than evidence of no difference) in test accuracy was detected between microarray-based and NGS-based cfDNA testing, including a head-to-head study which reported equivalent (perfect) test accuracy of the two approaches for the three

trisomies, and a meta-analysis of microarray-based cfDNA testing which produced comparable summary test accuracy estimates to NGS-based cfDNA testing in the previous review.[2,6]

The initial test failure rate for microarray-based cfDNA testing using the targeted DANSR approach is low, with summary initial test failure rate of 1.1% (95% CI 0.8% to 1.6%, four studies). Applicability issues of the underlying study populations that could affect the test failure rate (e.g. gestational age at testing, singleton or twin pregnancies, maternal weight/body mass index and the prevalence of fetal aneuploidy) need to be considered when transferring the findings to other populations. For comparison, summing across all nine studies using DANSR and NGS[14-22] as identified in our previous review[2,6] resulted in an initial test failure rate of 3.0% (933/31,077; 95% CI 2.7% to 3.3%). This comparison must be treated with caution as these were all published in an earlier time period and testing was performed in different populations.

The assessments of risk of bias and applicability of the studies using microarray-based cfDNA testing suggests that the evidence base is limited in terms of quality and generalisability. The key study by Juneau et al.[4] that directly compared test accuracy of NGS-based versus DNA microarray-based cfDNA testing was sponsored and performed by the manufacturer, and did not specify how samples for inclusion were chosen. This study therefore carries a high risk of bias.

The strengths of this systematic review are that we undertook a comprehensive search of the literature, with quality appraisal of all included studies, and two authors selecting studies for inclusion, extracting data and performing appraising quality. We synthesised findings in a meta-analysis. We also contacted study authors to clarify the used cfDNA methodologies and obtain subgroup data if needed.

Our review has a number of limitations. First, we restricted our search to English language papers; non-English language papers may be available and add further information. Second, we did not update the

evidence from the previous review on NGS-based cfDNA testing.[2,6] We therefore could not perform a valid indirect comparison of the test accuracy of microarray-based and NGS-based cfDNA test approaches. Third, many papers assessed for eligibility did not describe the cfDNA testing methodology used in sufficient detail to allow a decision on inclusion or exclusion. We therefore contacted the corresponding authors for clarification and realised that the majority of them sent the study samples to providers of commercially available cfDNA testing without knowing details of the testing method. For that reason, we contacted the laboratory concerned (Ariosa Diagnostics Inc., San Jose, CA) for information on the technologies used to analyse these particular study samples. Accuracy is therefore reliant on this information from the manufacturer. We received contradictory information for the cfDNA test methodology used in the study published by Miltoft et al. (2018)[23] and excluded it as Ariosa Diagnostics Inc. (San Jose, CA) confirmed that NGS was used for DNA quantitation in an unknown proportion of study samples. Fourth, we excluded seven studies (published in eight articles)[23-30] from this review that, according to information from Ariosa Diagnostics Inc. (San Jose, CA), used DNA microarray as DNA quantitation method in an unknown proportion of study samples. These 'mixed technology' studies might provide additional useful information on the test accuracy (see Appendix 5). Fifth, we did not consider the potential influence of test failures on the diagnostic performance of cfDNA testing in additional sensitivity analyses. The exclusion of test failures from the 2x2 tables might have led to the overestimation of sensitivity and specificity.[31] Finally, for the key head-to-head study,[4] we had to use unpublished data provided by the manufacturer for the subgroup of samples that had a suitable reference standard and this carries a risk of bias.

Conclusions

Our included studies suggest that NIPT using microarray-based cfDNA testing has high sensitivity and specificity for the detection of fetal trisomies 21, 18 and 13. However, the evidence base is currently small and at high risk of bias. No evidence of a difference in test accuracy between microarray- and NGS-

389 based approaches has been detected. We suggest a large, publicly funded population-based study on the

390 test accuracy and failure rate of microarray-based cfDNA testing in the first trimester.

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References

1. Gil MM, Quezada MS, Revello R*, et al.* Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol. 2015;45(3):249-266.

2. Taylor-Phillips S, Freeman K, Geppert J*, et al.* Accuracy of non-invasive prenatal testing using cell-free DNA for detection of Down, Edwards and Patau syndromes: a systematic review and meta-analysis. BMJ Open. 2016;6(1):e010002.

3. Badeau M, Lindsay C, Blais J*, et al.* Genomics-based non-invasive prenatal testing for detection of fetal chromosomal aneuploidy in pregnant women. Cochrane Database of Systematic Reviews. 2017;11:CD011767.

4. Juneau K, Bogard PE, Huang S*, et al.* Microarray-based cell-free DNA analysis improves noninvasive prenatal testing. Fetal Diagn Ther. 2014;36(4):282-286.

5. Bumgarner R. Overview of DNA microarrays: types, applications, and their future. Curr Protoc Mol Biol. 2013;Chapter 22:Unit 22 21.

6. Taylor-Phillips S, Freeman K, Geppert J*, et al.* Systematic review and cost-consequence assessment of cell-free DNA testing for T21, T18 and T13 in the UK – Final report. https://legacyscreening.phe.org.uk/fetalanomalies (Last external review - Systematic review: cfDNA); 2015.

7. Whiting PF, Rutjes AW, Westwood ME*, et al.* QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Annals of internal medicine. 2011;155(8):529-536.

8. Wilson EB. Probable Inference, the Law of Succession, and Statistical Inference. Journal of the American Statistical Association. 1927;22(158):209-212.

9. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. Stat Med. 1998;17(8):857-872.

10. Kagan KO, Sroka F, Sonek J*, et al.* First-trimester risk assessment based on ultrasound and cell-free DNA vs combined screening: a randomized controlled trial. Ultrasound in Obstetrics & Gynecology. 2018;51(4):437-444.

11. Langlois S, Johnson JA, Audibert F*, et al.* Comparison of first-tier cell-free DNA screening for common aneuploidies with conventional publically funded screening. Prenat Diagn. 2017;37(12):1238-1244.

12. Stokowski R, Wang E, White K*, et al.* Clinical performance of non-invasive prenatal testing (NIPT) using targeted cell-free DNA analysis in maternal plasma with microarrays or next generation sequencing (NGS) is consistent across multiple controlled clinical studies. Prenat Diagn. 2015;35(12):1243-1246.

13. Gil MM, Brik M, Casanova C*, et al.* Screening for trisomies 21 and 18 in a Spanish public hospital: from the combined test to the cell-free DNA test. Journal of Maternal-Fetal & Neonatal Medicine. 2017;30(20):2476-2482.

14. Sparks AB, Wang ET, Struble CA*, et al.* Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy. Prenat Diagn. 2012;32(1):3-9.

15. Sparks AB, Struble CA, Wang ET*, et al.* Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. Am J Obstet Gynecol. 2012;206(4):319 e311-319.

16. Norton ME, Jacobsson B, Swamy GK*, et al.* Cell-free DNA analysis for noninvasive examination of trisomy. N Engl J Med. 2015;372(17):1589-1597.

17. Willems PJ, Dierickx H, Vandenakker E*, et al.* The first 3,000 Non-Invasive Prenatal Tests (NIPT) with the Harmony test in Belgium and the Netherlands. Facts Views Vis Obgyn. 2014;6(1):7-12.

18. Ashoor G, Syngelaki A, Wagner M*, et al.* Chromosome-selective sequencing of maternal plasma cell-free DNA for first-trimester detection of trisomy 21 and trisomy 18. Am J Obstet Gynecol. 2012;206(4):322 e321-325.

19. Nicolaides KH, Syngelaki A, Ashoor G*, et al.* Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. Am J Obstet Gynecol. 2012;207(5):374 e371-376.

20. Norton ME, Brar H, Weiss J*, et al.* Non-Invasive Chromosomal Evaluation (NICE) Study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18. Am J Obstet Gynecol. 2012;207(2):137 e131-138.

21. Verweij EJ, Jacobsson B, van Scheltema PA*, et al.* European non-invasive trisomy evaluation (EU-NITE) study: a multicenter prospective cohort study for non-invasive fetal trisomy 21 testing. Prenat Diagn. 2013;33(10):996-1001.

22. Quezada MS, Gil MM, Francisco C*, et al.* Screening for trisomies 21, 18 and 13 by cell-free DNA analysis of maternal blood at 10-11 weeks' gestation and the combined test at 11-13 weeks. Ultrasound Obstet Gynecol. 2015;45(1):36-41.

23. Miltoft CB, Rode L, Ekelund CK*, et al.* Contingent first-trimester screening for aneuploidies with cell-free DNA in a Danish clinical setting. Ultrasound in Obstetrics & Gynecology. 2018;51(4):470-479.

24. Bevilacqua E, Jani JC, Letourneau A*, et al.* Cell-Free DNA Analysis in Maternal Blood: Differences in Estimates between Laboratories with Different Methodologies Using a Propensity Score Approach. Fetal Diagnosis and Therapy. 2018:1-10.

25. Bjerregaard L, Stenbakken AB, Andersen CS*, et al.* The rate of invasive testing for trisomy 21 is reduced after implementation of NIPT. Dan Med J. 2017;64(4).

26. Chan N, Smet ME, Sandow R*, et al.* Implications of failure to achieve a result from prenatal maternal serum cell-free DNA testing: a historical cohort study. BJOG: An International Journal of Obstetrics and Gynaecology. 2018;125(7):848-855.

27. Gil MM, Revello R, Poon LC*, et al.* Clinical implementation of routine screening for fetal trisomies in the UK NHS: cell-free DNA test contingent on results from first-trimester combined test. Ultrasound in Obstetrics & Gynecology. 2016;47(1):45-52.

28. Lee TJ, Rolnik DL, Menezes MA*, et al.* Cell-free fetal DNA testing in singleton IVF conceptions. Human Reproduction. 2018;33(4):572-578.

29. Revello R, Sarno L, Ispas A*, et al.* Screening for trisomies by cell-free DNA testing of maternal blood: consequences of a failed result. Ultrasound Obstet Gynecol. 2016;47(6):698-704.

30. Sarno L, Revello R, Hanson E*, et al.* Prospective first-trimester screening for trisomies by cell-free DNA testing of maternal blood in twin pregnancy. Ultrasound in Obstetrics & Gynecology. 2016;47(6):705-711.

31. Shinkins B, Thompson M, Mallett S*, et al.* Diagnostic accuracy studies: how to report and analyse inconclusive test results. BMJ. 2013;346:f2778.

Author contributions

JG conducted all aspects of the review and co-wrote the paper with CS.

CS conducted all aspects of the review and co-wrote the paper with JG.

AC undertook project planning and collaborated on research design. She read and commented on first and final drafts of the paper.

SJ developed and conducted the literature searches, managed references and helped in obtaining full text references.

DG advised the reviewers on the cfDNA test technology and commented on draft and final versions of the paper.

STP secured the funding, undertook project planning and research design, co-ordinated the review process, performed the meta-analyses and commented on draft and final versions of the paper.

JG, **CS**, **AC**, **SJ** and **STP** contributed to the development of the protocol.

Tables

Table 1. Main characteristics of included studies related to risk of bias and applicability concerns

Study	Design & setting	Patient selection	Population	Included in study	Exclusions from analyses	Included in cfDNA test accuracy analyses			
				n	n	Total (n)	T21 (n)	T18 (n)	T13 (n)
Gil 2017[13]	Uncontrolled before-after study; microarray-based cfDNA testing as follow-on test after FTCS; 1 University hospital in Spain.	Consecutive	Singleton pregnancies; 100% first trimester; only women with FTCS > 1:250 and without US anomalies were offered follow-on cfDNA testing.	6,011 (Before NIPT: 4,422; After NIPT: 1,589)	5,957/6,011 (99%): 5,939 not offered cfDNA testing; 18/72 (25%) did not choose cfDNA testing.	54	1	0	0
Juneau 2014[4]	Retrospective analysis of stored (frozen) blood samples; head-to-head comparison of microarray-based and NGS-based cfDNA testing; performed in-house by Ariosa Diagnostics Inc. (San Jose, CA).	Stored blood samples; enriched for trisomies, unclear how selected; exclusion of samples not meeting quality control for both quantitation methods.	Singleton pregnancies; mean 14.8 (SD 4.2) weeks' gestation, range 10-34 weeks' gestation; enriched for fetal trisomies.	878	486/878 (55%): 486 with unclear reference standard.	392	72	13	7
Kagan 2018[10]	Randomised controlled trial (1:1); FTCS versus US & cfDNA testing; 1 University hospital in Germany.	Consecutive women; randomised to FTCS or US & cfDNA testing.	Singleton pregnancies; 100% first trimester; low chance of fetal trisomies (NT ≤3.5mm and no fetal defects on US).	1,400 (699 FTCS, 701 US & cfDNA testing)	722/1,400 (52%): 699 randomised to FTCS, 13 without reference standard, 10 test failures.	678	0	0	0
Langlois 2017[11]	Prospective cohort study (substudy of PEGASUS study); head-to-head comparison of first-tier microarray-based cfDNA testing and conventional screening approaches; 3/5 centres from PEGASUS study: Vancouver, Calgary, Quebec (Canada).	Unclear	Singleton pregnancies; 100% first trimester; general obstetric population (no prior testing).	1,198	39/1,198 (3%): 6 test failures, 30 without reference standard, 3 wrong gestational dating.	1,159	6	0	0
Stokowski 2015[12]	Multicentre cohort study; retrospective analysis of stored (frozen) blood samples from Sweden, UK and USA; performed in-house by Ariosa Diagnostics Inc. (San Jose, CA).	Unclear, apparently enriched for trisomies.	759 singleton pregnancies, 40 twin pregnancies; Median 16 (IQR 13-19) weeks' gestation; apparently enriched for fetal trisomies.	799	8/799 (1%): 8 test failures.	791	108	30	12

cfDNA, cell-free deoxyribonucleic acid; FTCS, first trimester combined screening; IQR, interquartile range = 25th to 75th percentiles; NIPT, non-invasive prenatal testing; NT, nuchal translucency thickness; SD, standard deviation; T13, trisomy 13; T18, trisomy 18; T21, trisomy 21; US, ultrasound.

Figures

Figure 1. Workflow of different cfDNA test approaches. Marked in red is the step that is changed in microarray-based cfDNA testing.

Figure 2. PRISMA flow diagram of records through the systematic review.

† In seven studies (published in eight articles) the provider of the commercially available cfDNA test used both sequencing as well as DNA microarray technologies to analyse the study samples in unclear proportions. In one of these studies, the corresponding author confirmed the use of the microarray-based cfDNA test version for all samples while the provider of the cfDNA test analyses claimed that both test versions were used. These 'mixed technology' studies were excluded from our review; study details and relevant findings are presented in Appendix 5.

Figure 3. Sensitivity (%) and specificity (%) of prenatal cfDNA testing with DNA microarray for the detection of fetal trisomy 21 (T21), trisomy 18 (T18) and trisomy 13 (T13) in individual studies.

Figure 4. Findings for microarray-based cfDNA testing applied to a hypothetical cohort of 10,000 pregnancies at higher chance of fetal trisomy (95% confidence intervals in brackets). Confidence intervals reflect statistical uncertainty only; accuracy may be overestimated due to high risk of bias of included studies and exclusion of test failures. Initial test failure rate in this first-trimester, higher-chance population might have been underestimated due to applicability issues in the underlying studies. Women with initial cfDNA test failure might be at increased chance of fetal aneuploidy and should be offered further testing and counselling.

Appendices

Appendix 1. Summary of electronic database searches and search dates.

Appendix 2. Search strategies.
A - Medline (Ovid)
B - Medline In-Process & Other Non-Indexed Citations (Ovid)
C - Embase (Ovid)
D - Cochrane Library (Wiley)
E - Web of Science (Science and Social Science databases, Science and Social Sciences Conferences) (Clarivate Analytics)

Appendix 3. Publications excluded after review of full-text articles, with reason (n=133).

Appendix 4. Information about author contacts and replies.

Appendix 5. Details of excluded studies using cfDNA testing with both sequencing and DNA microarray technologies (7 'mixed technology' studies published in 8 articles).

Appendix 6. Modifications to the standard QUADAS-2 tool.[7]

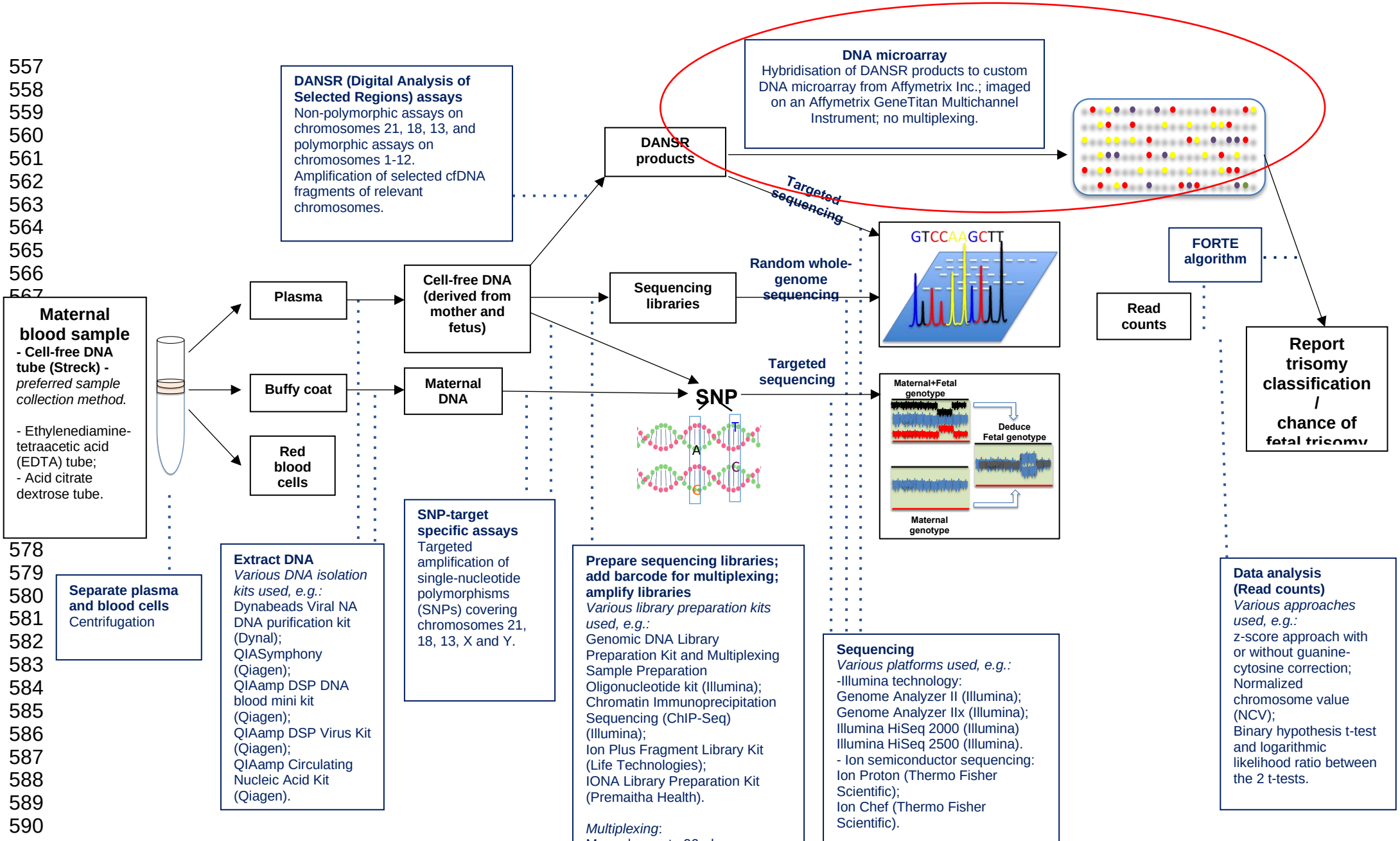
Appendix 7. Tailored QUADAS-2 tool[7] and guidance notes.

Appendix 8. Study level data.

Appendix 9. Quality assessment of all included studies (n=5) using the tailored QUADAS-2 tool. [7]
A - Individual study ratings.
B - Proportion of studies with low, high and unclear risk of bias for each QUADAS-2 domain.
C - Proportion of studies with low, high and unclear applicability concerns for each QUADAS-2 domain.

Appendix 10. Test accuracy outcomes in individual studies (n=5).

Appendix 11. Initial test failure rates, reasons for failure and success of repeat tests (4 studies).


DANSR (Digital Analysis of Selected Regions) assays
Non-polymorphic assays on chromosomes 21, 18, 13, and polymorphic assays on chromosomes 1-12.
Amplification of selected cfDNA fragments of relevant chromosomes.
DNA microarray
Hybridisation of DANSR products to custom DNA microarray from Affymetrix Inc.; imaged on an Affymetrix GeneTitan Multichannel Instrument; no multiplexing.
DANSR products
Targeted sequencing
Maternal blood sample
- Cell-free DNA tube (Streck) - preferred sample collection method.
- Ethylenediamine-tetraacetic acid (EDTA) tube;
- Acid citrate dextrose tube.
Plasma
Buffy coat
Red blood cells
Cell-free DNA (derived from mother and fetus)
Maternal DNA
Sequencing libraries
Random whole-genome sequencing
Targeted sequencing
SNP
GTCCAAGCTT
Maternal+Fetal genotype
Deduce Fetal genotype
Maternal genotype
FORTE algorithm
Read counts
Report trisomy classification / chance of fetal trisomy
Separate plasma and blood cells
Centrifugation
Extract DNA
Various DNA isolation kits used, e.g.:
Dynabeads Viral NA DNA purification kit (Dynal);
QIASymphony (Qiagen);
QIAamp DSP DNA blood mini kit (Qiagen);
QIAamp DSP Virus Kit (Qiagen);
QIAamp Circulating Nucleic Acid Kit (Qiagen).
SNP-target specific assays
Targeted amplification of single-nucleotide polymorphisms (SNPs) covering chromosomes 21, 18, 13, X and Y.
Prepare sequencing libraries; add barcode for multiplexing; amplify libraries
Various library preparation kits used, e.g.:
Genomic DNA Library Preparation Kit and Multiplexing Sample Preparation Oligonucleotide kit (Illumina);
Chromatin Immunoprecipitation Sequencing (ChIP-Seq) (Illumina);
Ion Plus Fragment Library Kit (Life Technologies);
IONA Library Preparation Kit (Premaitha Health).
Multiplexing:
Monoplex up to 96-plex.
Amplification: Different number of PCR cycles.
Sequencing
Various platforms used, e.g.:
-Illumina technology:
Genome Analyzer II (Illumina);
Genome Analyzer IIx (Illumina);
Illumina HiSeq 2000 (Illumina)
Illumina HiSeq 2500 (Illumina).
- Ion semiconductor sequencing:
Ion Proton (Thermo Fisher Scientific);
Ion Chef (Thermo Fisher Scientific).
Other differences:
Read lengths;
Paired-end vs single end;
Human reference genome;
Alignment software;
Mismatches allowed.
Data analysis (Read counts)
Various approaches used, e.g.:
z-score approach with or without guanine-cytosine correction;
Normalized chromosome value (NCV);
Binary hypothesis t-test and logarithmic likelihood ratio between the 2 t-tests.

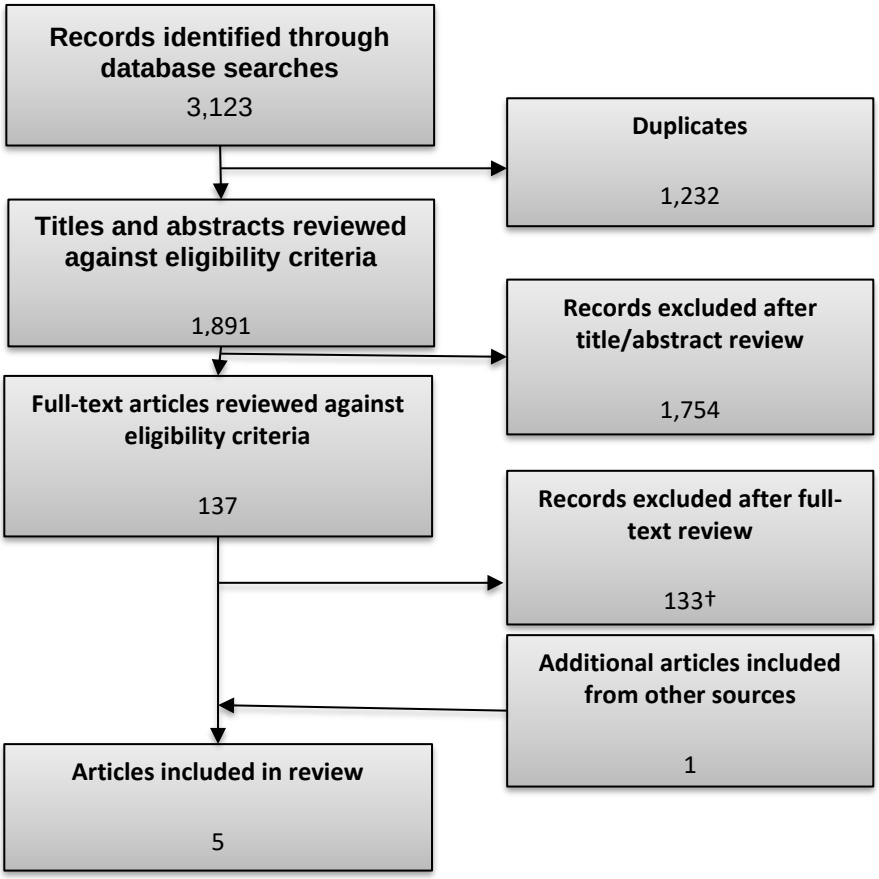


Figure 2. PRISMA flow diagram of records through the systematic review.

† In seven studies (published in eight articles) the provider of the commercially available cfDNA test used both sequencing as well as DNA microarray technologies to analyse the study samples in unclear proportions. In one of these studies, the corresponding author confirmed the use of the microarray-based cfDNA test version for all samples while the provider of the cfDNA test analyses claimed that both test versions were used. These 'mixed technology' studies were excluded from our review; study details and relevant findings are presented in Appendix 5.

STUDY	TP	FP	FN	TN	% sensitivity [95% CI]	% specificity [95% CI]
Microarray-T21						
Gil 2017	1	0	0	53	100 [5.46-100]	100 [91.6 -100]
Juneau 2014	72	0	0	320	100 [93.7 -100]	100 [98.5 -100]
Kagan 2018	0	0	0	678	not estimable	100 [99.3-100]
Langlois 2017	6	0	0	1153	100 [51.7 -100]	100 [99.6-100]
Stokow ski 2015	107	0	1	683	99.1 [94.2-99.95]	100 [99.3-100]
Microarray-T18						
Gil 2017	0	0	0	54	not estimable	100 [91.7-100]
Juneau 2014	13	0	0	379	100 [71.7 -100]	100 [98.7 -100]
Kagan 2018	0	0	0	678	not estimable	100 [99.3 -100]
Langlois 2017	0	1	0	1158	not estimable	99.9 [99.4-100]
Stokow ski 2015	29	0	1	761	96.7 [80.9-99.8]	100 [99.4-100]
Microarray-T13						
Gil 2017	0	0	0	54	not estimable	100 [91.7-100]
Juneau 2014	7	0	0	385	100 [56.1-100]	100 [98.8 -100]
Kagan 2018	0	0	0	678	not estimable	100 [99.3-100]
Langlois 2017	0	1	0	1158	not estimable	99.9 [99.4-100]
Stokow ski 2015	12	0	0	779	100 [69.9 -100]	100 [99.4-100]

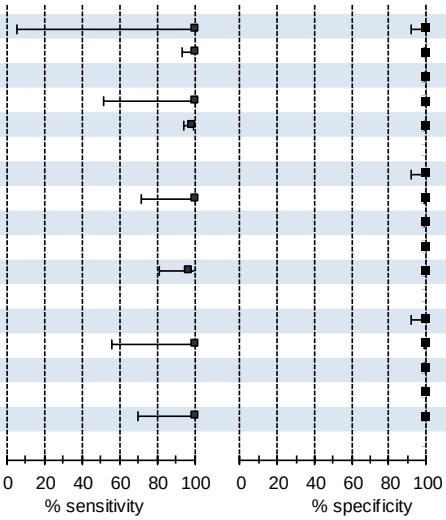


Figure 3. Sensitivity (%) and specificity (%) of prenatal cfDNA testing with DNA microarray for the detection of fetal trisomy 21 (T21), trisomy 18 (T18) and trisomy 13 (T13) in individual studies.

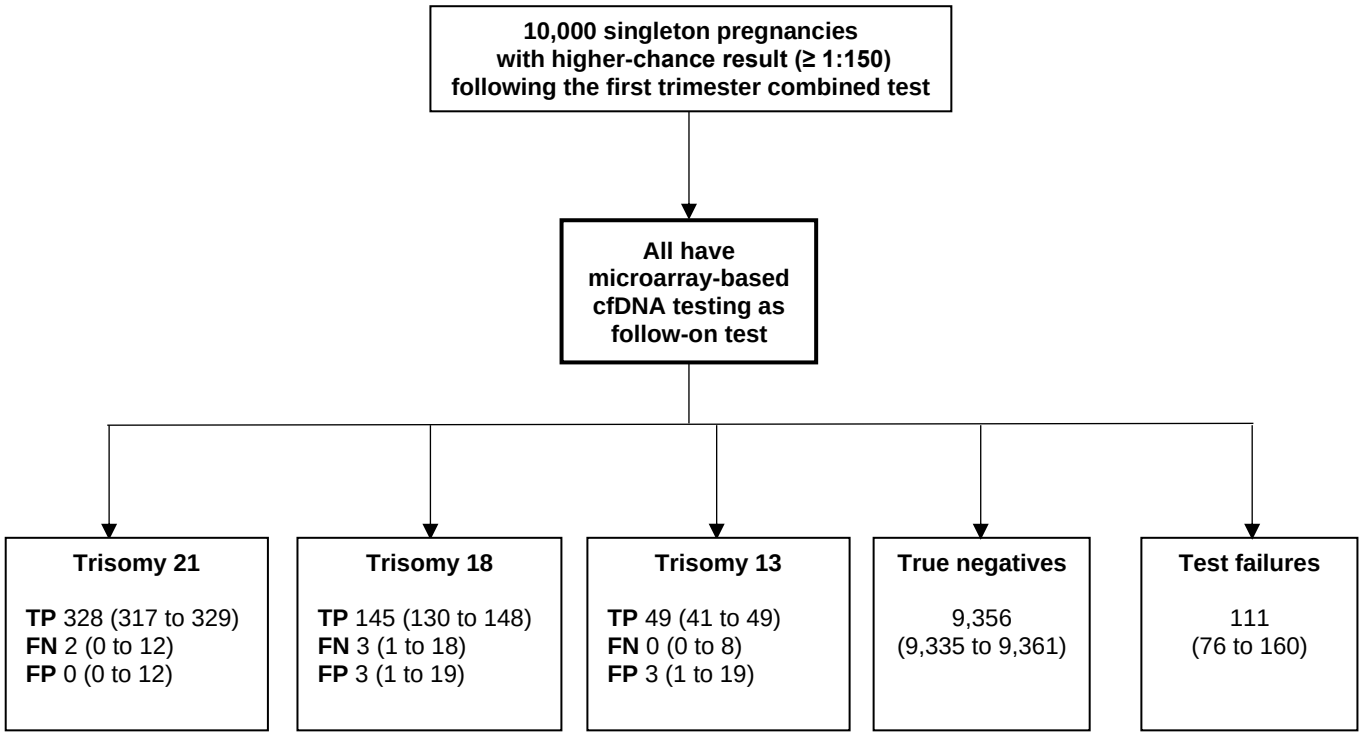


Figure 4. Findings for microarray-based cfDNA testing applied to a hypothetical cohort of 10,000 pregnancies at higher chance of fetal trisomy (95% confidence intervals in brackets). Confidence intervals reflect statistical uncertainty only; accuracy may be overestimated due to high risk of bias of included studies and exclusion of test failures. Initial test failure rate in this first-trimester, higher-chance population might have been underestimated due to applicability issues in the underlying studies. Women with initial cfDNA test failure might be at increased chance of fetal aneuploidy and should be offered further testing and counselling.

Appendix 1. Summary of electronic database searches and search dates.

Database	Platform	Searched on date	Date range of search
MEDLINE	Ovid SP	9th July 2018	February 2015 to July 2018
MEDLINE In-Process, MEDLINE Daily, Epub Ahead of Print	Ovid SP	9th July 2018	February 2015 to July 2018
Embase	Ovid SP	9th July 2018	February 2015 to July 2018
The Cochrane Library, including: - **Cochrane Database of Systematic Reviews (CDSR)** - **Cochrane Central Register of Controlled Trials (CENTRAL)** - **Database of Abstracts of Reviews of Effects (DARE)**	Wiley Online	9th July 2018	February 2015 to July 2018
Web of Science	Clarivate Analytics	9th July 2018	Years 2015 - 2018

Appendix 2. Search strategies
A - Search strategy for Medline (Ovid)
Database: Ovid MEDLINE(R) <1946 to June Week 5 2018>
Search Strategy:

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1 ((noninvasive or non-invasive or non invasive) adj3 (prenatal or pre?natal* or pregnanc* or diagnos* or test* or detect* or screen* or assess*)).mp. (29480)

2 (NIPD or NIPT).mp. (482)

3 (cf?DNA or cff?DNA or ccff?DNA or cell?free?DNA).mp. (782)

4 (DNA adj1 (cell or free or cell?free or f?etal)).mp. (8908)

5 (maternal adj1 (blood or plasma or DNA)).mp. (10377)

6 (MPS or DANSR or parental support or MaterniT21 or Verify or Harmony or Panorama*).mp. (59679)

7 1 or 2 or 3 or 4 or 5 or 6 (106210)

8 Trisomy/ (11695)

9 trisom*.mp. (20398)

10 Aneuploidy/ (11637)

11 aneuploid*.mp. (21616)

12 Down Syndrome/ (23063)

13 (down* adj1 syndrom*).mp. (26889)

14 (edward* adj1 syndrom*).mp. (269)

15 (Patau adj1 syndrom*).mp. (134)

16 ("T21" or "T18" or "T13").mp. (1325)

17 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 (59578)

18 7 and 17 (1593)

19 limit 18 to ed=20150209-20180709 (607)

20 limit 19 to english language (551)

B - Search strategy for Medline In-Process & Other Non-Indexed Citations (Ovid)

Database: Ovid MEDLINE(R) Daily Update <July 06, 2018>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations <July 06, 2018>, Ovid MEDLINE(R) Epub Ahead of Print <July 06, 2018>

Search Strategy:

1 ((noninvasive or non-invasive or non invasive) adj3 (prenatal or pre?natal* or pregnanc* or diagnos* or test* or detect* or screen* or assess*)).mp. (4198)

2 (NIPD or NIPT).mp. (224)

3 (cf?DNA or cff?DNA or ccff?DNA or cell?free?DNA).mp. (409)

4 (DNA adj1 (cell or free or cell?free or f?etal)).mp. (1195)

5 (maternal adj1 (blood or plasma or DNA)).mp. (769)

6 (MPS or DANSR or parental support or MaterniT21 or Verify or Harmony or Panorama*).mp. (14682)

7 1 or 2 or 3 or 4 or 5 or 6 (20494)

8 Trisomy/ (5)

9 trisom*.mp. (1213)

10 Aneuploidy/ (3)

11 aneuploid*.mp. (1575)

12 Down Syndrome/ (18)

13 (down* adj1 syndrom*).mp. (1587)

14 (edward* adj1 syndrom*).mp. (27)

15 (Patau adj1 syndrom*).mp. (25)

16 ("T21" or "T18" or "T13").mp. (191)

17 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 (3798)

18 7 and 17 (293)

19 limit 18 to ed=20150209-20180709 (41)

20 limit 19 to english language (40)

C - Search strategy for Embase (Ovid)
Database: Embase <1980 to 2018 Week 28>
Search Strategy:

1 ((noninvasive or non-invasive or non invasive) adj3 (prenatal or pre?natal* or pregnanc* or diagnos* or test* or detect* or screen* or assess*)).mp. (49472)

2 (NIPD or NIPT).mp. (1240)

3 (cf?DNA or cff?DNA or ccff?DNA or cell?free?DNA).mp. (2627)

4 (DNA adj1 (cell or free or cell?free or f?etal)).mp. (23379)

5 (maternal adj1 (blood or plasma or DNA)).mp. (17543)

6 (MPS or DANSR or parental support or MaterniT21 or Verifi* or Harmony or Panorama*).mp. (173918)

7 1 or 2 or 3 or 4 or 5 or 6 (259158)

8 Trisomy/ (9503)

9 trisom*.mp. (29540)

10 Aneuploidy/ (21780)

11 aneuploid*.mp. (30257)

12 Down Syndrome/ (30581)

13 (down* adj1 syndrom*).mp. (34020)

14 (edward* adj1 syndrom*).mp. (665)

15 (Patau adj1 syndrom*).mp. (321)

16 ("T21" or "T18" or "T13").mp. (2184)

17 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 (82880)

18 7 and 17 (3909)

19 limit 18 to english language (3617)

20 limit 19 to dc=20150209-20180709 (1454)

21 limit 19 to em=201502-201828 (1203)

22 20 or 21 (1503)

D - Search strategy for Cochrane Library (Wiley)

#1	((noninvasive or non-invasive or non invasive) near/3 (prenatal or pre?natal* or pregnanc* or diagnos* or test* or detect* or screen* or assess*)):ti,ab,kw or (NIPD or NIPT):ti,ab,kw or (cfDNA or cffDNA or ccffDNA or "cell free DNA"):ti,ab,kw or (DNA near/3 (cell or free or cell?free or f?etal)):ti,ab,kw or (maternal near/3 (blood or plasma or DNA)):ti,ab,kw (Word variations have been searched)	S	3549
#2	(MPS or DANSR or parental support or MaterniT21 or Verify or Harmony or Panorama*):ti,ab,kw (Word variations have been searched)	S	9706
#3	#1 or #2		13211
#4	MeSH descriptor: [Trisomy] explode all trees	M	31
#5	trisom*:ti,ab,kw (Word variations have been searched)	S	237
#6	MeSH descriptor: [Aneuploidy] explode all trees	M	170
#7	Aneuploid*:ti,ab,kw (Word variations have been searched)	S	391
#8	MeSH descriptor: [Down Syndrome] explode all trees	M	420
#9	(down* near/1 syndrom*):ti,ab,kw (Word variations have been searched)	S	674
#10	(edward* near/1 syndrom*):ti,ab,kw (Word variations have been searched)	S	5
#11	(patau near/1 syndrom*):ti,ab,kw (Word variations have been searched)	S	1
#12	t21 or t18 or t13:ti,ab,kw (Word variations have been searched)	S	48
#13	#4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12		1251
#14	#3 and #13		113

E - Search strategy for Web of Science (Science and Social Science databases, Science and Social Sciences Conferences) (Clarivate Analytics)

# 5	987	#2 AND #1 **Refined by: PUBLICATION YEARS:** (2018 OR 2017 OR 2016 OR 2015) AND **LANGUAGES:** (ENGLISH) *Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI Timespan=All years*
# 4	1,017	#2 AND #1 **Refined by: PUBLICATION YEARS:** (2018 OR 2017 OR 2016 OR 2015) *Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI Timespan=All years*
# 3	3,434	#2 AND #1 *Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI Timespan=All years*
# 2	58,737	**TOPIC:** (trisom* or aneuploid* or "down syndrome") *OR* **TOPIC:** (down NEAR/1 syndrom*) *OR* **TOPIC:** (edward* NEAR/1 syndrom*) *OR* **TOPIC:** (patau* NEAR/1 syndrom*) *OR* **TOPIC:** (T21 or T18 or T13) *Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI Timespan=All years*
# 1	621,942	**TOPIC:** (((noninvasive or non-invasive or "non invasive") near/3 (prenatal or pre?natal* or pregnanc* or diagnos* or test* or detect* or screen* or assess*))) *OR* **TOPIC:** ((NIPD or NIPT)) *OR* **TOPIC:** ((cfDNA or cffDNA or ccffDNA or "cell free DNA")) *OR* **TOPIC:** ((DNA near/3 (cell or free or cell?free or f?etal))) *OR* **TOPIC:** ((maternal near/3 (blood or plasma or DNA))) *OR* **TOPIC:** ((MPS or DANSR or "parental support" or MaterniT21 or Verifi* or Harmony or Panorama)) *Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI Timespan=All years*

Appendix 3. Publications excluded after review of full-text articles, with reason (n=133)

Reference		Reason for exclusion
1.	Barbu, M., et al. (2017). First Trimester Screening Options after the Introduction of NIPT - our Experience.	Exclude as full text not available. Conference paper (5th Romanian Congress of the Romanian Society of Ultrasound in Obstetrics and Gynecology).
2.	Bayindir, B., et al. (2015). "Noninvasive prenatal testing using a novel analysis pipeline to screen for all autosomal fetal aneuploidies improves pregnancy management." European Journal of Human Genetics 23(10): 1286-1293.	Exclude as sequencing not microarray for DNA quantification.
3.	Belloin, C., et al. (2016). "The noninvasive prenatal testing for Down's Syndrome. Retrospective study of 8821 patients." Journal de Gynecologie Obstetrique et Biologie de la Reproduction 45(9): 1127-1132.	Exclude as in French language.
4.	Benachi, A., et al. (2015). "Cell-free DNA analysis in maternal plasma in cases of fetal abnormalities detected on ultrasound examination." Obstetrics & Gynecology 125(6): 1330-1337.	Exclude as MPSS not microarray.
5.	Benn, P. and F. R. Grati (2018). "Genome-wide non-invasive prenatal screening for all cytogenetically visible imbalances." Ultrasound in Obstetrics & Gynecology 51(4): 429-433	Exclude as editorial.
6.	Bestwick, J. P. and N. J. Wald (2016). "Antenatal reflex DNA screening for trisomy 18 and trisomy 13 in addition to Down's syndrome." Journal of Medical Screening 23(4): 171-174.	Exclude as simulation/modelling.
7.	Beulen, L., et al. (2017). "Clinical utility of non-invasive prenatal testing in pregnancies with ultrasound anomalies." Ultrasound in Obstetrics & Gynecology 49(6): 721-728.	Exclude as MPSS was used not microarray.
8.	Bevilacqua, E., et al. (2018). "Cell-Free DNA Analysis in Maternal Blood: Differences in Estimates between Laboratories with Different Methodologies Using a Propensity Score Approach." Fetal Diagnosis and Therapy: 1-10.	This study compares a MPSS-based cfDNA test to the Harmony Prenatal Test. Exclude as Ariosa/Roche confirmed the use of both sequencing and microarray approaches in unclear proportions.
9.	Bevilacqua, E., et al. (2017). "Screening for Sex Chromosome Aneuploidy by Cell-Free DNA Testing: Patient Choice and Performance." Fetal Diagnosis and Therapy. 23.	Exclude as Harmony Prenatal test was used, unclear from the publication if microarray technology, no test performance data for T21, T18 and T13. Ariosa/Roche confirmed the use of both sequencing and microarray approaches in unclear proportions.
10.	Bianchi, D. W., et al. (2015). "Noninvasive Prenatal Testing and Incidental Detection of Occult Maternal Malignancies.[Summary for patients in JAMA. 2015 Jul 14;314(2):198; PMID: 26172909]." JAMA 314(2): 162-169.	Exclude as MPSS was used, not microarray.

Reference		Reason for exclusion
11.	Bianchi, D. W., et al. (2015). "Fetal sex chromosome testing by maternal plasma DNA sequencing: clinical laboratory experience and biology." Obstetrics & Gynecology 125(2): 375-382.	Exclude as MPSS methodology was used, not microarray.
12.	Bjerregaard, L., et al. (2017). "The rate of invasive testing for trisomy 21 is reduced after implementation of NIPT." Danish Medical Journal 64(4).	Harmony Prenatal Test was used, but unclear from publication if sequencing or microarray technology. Study period: 1 March 2013 to 1 February 2015. Exclude as Ariosa/Roche confirmed that Harmony Prenatal Test with both sequencing and microarray approach was used for analysis.
13.	Blackwell, S., et al. (2015). "#36: Prenatal aneuploidy screening using cell-free DNA." American Journal of Obstetrics and Gynecology 212(6): 711-716.	Exclude as no primary research article.
14.	Brison, N., et al. (2018). "Predicting fetoplacental chromosomal mosaicism during non-invasive prenatal testing." Prenatal Diagnosis 38(4): 258-266.	Exclude as MPSS not microarray.
15.	Chitty, L. S., et al. (2016). "Uptake, outcomes, and costs of implementing non-invasive prenatal testing for Down's syndrome into NHS maternity care: prospective cohort study in eight diverse maternity units." BMJ 354: i3426.	Exclude as sequencing-based, not microarray-based NIPT.
16.	Chu, T., et al. (2017). "Comparative evaluation of the Minimally-Invasive Karyotyping (MINK) algorithm for non-invasive prenatal testing." PLoS ONE [Electronic Resource] 12(3): e0171882.	Exclude as MPSS not microarray.
17.	Cirigliano, V., et al. (2017). "Performance of the neoBona test: a new paired-end massively parallel shotgun sequencing approach for cell-free DNA-based aneuploidy screening." Ultrasound in Obstetrics & Gynecology 49(4): 460-464.	Exclude as MPSS-based NIPT, not microarray.
18.	Crea, F., et al. (2017). "The IONA Test: Development of an Automated Cell-Free DNA-Based Screening Test for Fetal Trisomies 13, 18, and 21 That Employs the Ion Proton Semiconductor Sequencing Platform." Fetal Diagnosis & Therapy 42(3): 218-224.	Exclude as NGS on an Ion Proton sequencing platform, not microarray.
19.	Dahl, F., et al. (2018). "Imaging single DNA molecules for high precision NIPT." Scientific Reports 8.	Exclude as no DNA microarray was used: novel molecular probe technology.
20.	Dheedene, A., et al. (2016). "Implementation of non-invasive prenatal testing by semiconductor sequencing in a genetic laboratory." Prenatal Diagnosis 36(8): 699-707.	Exclude as Ion Proton sequencing not microarray.
21.	Dobson, L. J., et al. (2016). "Patient choice and clinical outcomes following positive noninvasive prenatal screening for aneuploidy with cell-free DNA (cfDNA)." Prenatal Diagnosis 36(5): 456-462.	Exclude as "The commercial enterprise performing the cfDNA was at the discretion of the obstetrical provider and represented three different companies." Unclear if microarray, but no separate data available.
22.	Du, E., et al. (2017). "Massively Parallel Sequencing (MPS) of Cell-Free Fetal DNA (cffDNA) for Trisomies 21, 18, and 13 in Twin Pregnancies." Twin Research & Human Genetics: the Official Journal of the International Society for Twin Studies 20(3): 242-249.	Exclude as MPS testing not microarray.

Reference		Reason for exclusion
23.	Ehrich, M., et al. (2017). "Genome-wide cfDNA screening: Clinical laboratory experience with the first 10,000 cases." Genetics in Medicine 19(12): 1332-1337.	Exclude as whole genome sequencing, not microarray.
24.	Eiben, B., et al. (2015). "Single Nucleotide Polymorphism-Based Analysis of Cell-Free Fetal DNA in 3000 Cases from Germany and Austria." Ultrasound International Open 1(1): E8-E11.	Exclude as SNP-based sequencing (Natera) not microarray.
25.	El Khattabi, L. A., et al. (2016). "Could Digital PCR Be an Alternative as a Non-Invasive Prenatal Test for Trisomy 21: A Proof of Concept Study." PLoS ONE [Electronic Resource] 11(5): e0155009.	Exclude as digital PCR not microarray.
26.	Ellison, C. K., et al. (2016). "Using Targeted Sequencing of Paralogous Sequences for Noninvasive Detection of Selected Fetal Aneuploidies." Clinical Chemistry 62(12): 1621-1629.	Exclude as sequencing not microarray.
27.	Fiorentino, F., et al. (2017). "The clinical utility of genome-wide non invasive prenatal screening." Prenatal Diagnosis 37(6): 593-601.	Exclude as sequencing not microarray.
28.	Flock, A., et al. (2017). "Non-invasive prenatal testing (NIPT): Europe's first multicenter post-market clinical follow-up study validating the quality in clinical routine." Archives of Gynecology & Obstetrics 296(5): 923-928.	Exclude as random massively parallel sequencing, not microarray.
29.	Fosler, L., et al. (2017). "Aneuploidy screening by non-invasive prenatal testing in twin pregnancy." Ultrasound in Obstetrics & Gynecology 49(4): 470-477.	Exclude as sequencing not microarray.
30.	Gerundino, F., et al. (2017). "Validation of a method for noninvasive prenatal testing for fetal aneuploidies risk and considerations for its introduction in the Public Health System." Journal of Maternal-Fetal & Neonatal Medicine 30(6): 710-716.	Exclude as whole-genome MPS-based NIPT method not microarray.
31.	Gil, M. M., et al. (2016). "Clinical implementation of routine screening for fetal trisomies in the UK NHS: cell-free DNA test contingent on results from first-trimester combined test." Ultrasound in Obstetrics & Gynecology 47(1): 45-52.	Harmony Prenatal Test was used; unclear from publication if with sequencing or microarray technology. Study period: October 2013 and February 2015. Exclude as Ariosa/Roche confirmed the use of both NGS and microarray approaches in unclear proportions.
32.	Gill, L. A. and T. L. Prosen (2017). "Indications for Invasive Prenatal Testing before and after Noninvasive Prenatal Screening." American Journal of Perinatology 34(11): 1084-1087.	Exclude as no test accuracy/failure data reported, no information on NIPT methodology used.
33.	Gomez-Manjon, I., et al. (2018). "Noninvasive Prenatal Testing: Comparison of Two Mappers and Influence in the Diagnostic Yield." BioMed Research International 2018 (no pagination)(9498140).	Exclude as MPSS not microarray.
34.	Hartwig, T. S., et al. (2018). "Non-Invasive Prenatal Testing (NIPT) in pregnancies with trisomy 21, 18 and 13 performed in a public setting - factors of importance for correct interpretation of results." European Journal of Obstetrics Gynecology and Reproductive Biology 226: 35-39.	Exclude as MPS not microarray-based NIPT.
35.	Hu, H., et al. (2016). "Clinical Experience of Non-Invasive Prenatal Chromosomal Aneuploidy Testing in 190,277 Patient Samples." Current Molecular Medicine 16(8): 759-766.	Exclude as semiconductor sequencing was used, not microarray.

Reference		Reason for exclusion
36.	Huang, S., et al. (2016). "Identifying Robertsonian Translocation Carriers by Microarray-Based DNA Analysis." Fetal Diagnosis & Therapy 40(1): 59-62.	Though it used microarray, exclude as case control study with only 7 pregnant cases; maternal Robertsonian translocation carriers, not T21/Robertsonian translocation in the fetus.
37.	Johansen, P., et al. (2016). "Open source non-invasive prenatal testing platform and its performance in a public health laboratory." Prenatal Diagnosis 36(6): 530-536.	Exclude as whole-genome sequencing on the Ion Proton™ platform, not microarray.
38.	Jones, K. J., et al. (2018). "Targeted cell-free DNA analysis with microarray quantitation for assessment of fetal sex and sex chromosome aneuploidy risk." Ultrasound in Obstetrics & Gynecology 51(2): 275-+.	Exclude as letter.
39.	Kagan, K. O., et al. (2015). "Screening for chromosomal abnormalities by first trimester combined screening and noninvasive prenatal testing." Ultraschall in der Medizin 36(1): 40-46.	Exclude as modelling study, no NIPT performed, just assumed performance from the literature.
40.	Kane, S. C., et al. (2017). "Chorionic villus sampling in the cell-free DNA aneuploidy screening era: careful selection criteria can maximise the clinical utility of screening and invasive testing." Prenatal Diagnosis 37(4): 399-408.	Exclude as unclear if NIPT methodology involved microarray.
41.	Ke, W.-L., et al. (2015). "Detection of fetal cell-free DNA in maternal plasma for Down syndrome, Edward syndrome and Patau syndrome of high risk fetus." International journal of clinical and experimental medicine 8(6): 9525-9530.	Exclude as sequencing not microarray.
42.	Kim, S., et al. (2016). "Comparison of two high-throughput semiconductor chip sequencing platforms in noninvasive prenatal testing for Down syndrome in early pregnancy." BMC Medical Genomics [Electronic Resource] 9(1): 22.	Exclude as semiconductor sequencing comparing Ion Torrent PGM and Proton platforms.
43.	Kim, S., et al. (2016). "An adaptive detection method for fetal chromosomal aneuploidy using cell-free DNA from 447 Korean women." BMC Medical Genomics [Electronic Resource] 9(1): 61.	Exclude as sequencing not microarray was used.
44.	Kornman, L., et al. (2017). "Non-Invasive Prenatal Testing for Sex Chromosome Aneuploidy in Routine Clinical Practice." Fetal Diagnosis and Therapy. 06.	Publication states that the Harmony Prenatal Test was used with reference to the method published in Sparks (2012), which is based on targeted sequencing not microarray. Study period: March 2013 and August 2014. Agreed to exclude as study period was before 10th November 2014 which was the date confirmed by Ariosa/Roche on which they started running their samples using the microarray platform.
45.	Kou, K. O., et al. (2016). "Effect of non-invasive prenatal testing as a contingent approach on the indications for invasive prenatal diagnosis and prenatal detection rate of Down's syndrome." Hong Kong Medical Journal 22(3): 223-230.	Exclude as no mention of microarray, just "shotgun" and "targeted" DNA sequencing.

Reference		Reason for exclusion
46.	Koumbaris, G., et al. (2016). "Cell-Free DNA Analysis of Targeted Genomic Regions in Maternal Plasma for Non-Invasive Prenatal Testing of Trisomy 21, Trisomy 18, Trisomy 13, and Fetal Sex." Clinical Chemistry 62(6): 848-855.	Exclude as targeted sequencing was used, not microarray.
47.	Krishna, I., et al. (2016). "Adverse perinatal outcomes are more frequent in pregnancies with a low fetal fraction result on noninvasive prenatal testing." Prenatal Diagnosis 36(3): 210-215.	Exclude as targeted sequencing or SNP sequencing was used; not microarray.
48.	Le Conte, G., et al. (2018). "Cell-free fetal DNA analysis in maternal plasma as a screening test for trisomy 21 in twin pregnancies." Gynecologie Obstetrique Fertilite et Senologie.	Exclude as article in French language.
49.	Lebo, R. V., et al. (2015). "Discordant circulating fetal DNA and subsequent cytogenetics reveal false negative, placental mosaic, and fetal mosaic cfDNA genotypes." Journal of Translational Medicine 13: 260.	Exclude as NIPT performed at (1) Sequenom testing with MaterniT21, or (2) Ariosa Diagnostics testing with Harmony at Integrated Genetics. Unclear if microarray technology.
50.	Lee, S. Y., et al. (2018). "A new approach of digital PCR system for non-invasive prenatal screening of trisomy 21." Clinica Chimica Acta 476: 75-80.	Exclude as NIPT using digital PCR not microarray for DNA quantification.
51.	Lee, S. Y., et al. (2015). "New application methods for chromosomal abnormalities screening test using digital PCR." Biochip Journal 9(4): 339-352.	Exclude as case control study with <15 T21 cases; digital PCR, not microarray, used for cfDNA quantification.
52.	Lee, M.-Y., et al. (2015). "Performance of Momguard, a new non-invasive prenatal testing protocol developed in Korea." Obstetrics & Gynecology Science 58(5): 340-345.	Exclude as sequencing not microarray-based NIPT.
53.	Lee, T. J., et al. (2018). "Cell-free fetal DNA testing in singleton IVF conceptions." Human Reproduction 33(4): 572-578.	Harmony Prenatal Test was used with reference to Sparks (2012) method which is based on targeted sequencing not microarray. Study period: April 2013 and November 2016. Exclude as Ariosa/Roche confirmed that Harmony Prenatal Test with both sequencing and microarray approach was used for analysis.
54.	Lefkowitz, R. B., et al. (2016). "Clinical validation of a noninvasive prenatal test for genomewide detection of fetal copy number variants." American Journal of Obstetrics & Gynecology 215(2): 227.e221-227.e216.	Exclude as sequencing, not microarray-based NIPT.
55.	Li, B., et al. (2016). "Applicability of first-trimester combined screening for fetal trisomy 21 in a resource-limited setting in mainland China." BJOG: An International Journal of Obstetrics and Gynaecology 123(Supplement 3): 23-29.	Exclude as cfDNA test methodology not reported.
56.	Li, R., et al. (2016). "Detection of fetal copy number variants by non-invasive prenatal testing for common aneuploidies." Ultrasound in Obstetrics & Gynecology 47(1): 53-57.	Exclude as semiconductor sequencing (MPSS), not microarray-based NIPT.
57.	Li, S. W., et al. (2015). "The assessment of combined first trimester screening in women of advanced maternal	Exclude as no NIPT performed.

Reference		Reason for exclusion
	age in an Asian cohort." Singapore Medical Journal 56(1): 47-52.	
58.	Li, W. H., et al. (2015). "Noninvasive prenatal testing for fetal trisomy in a mixed risk factors pregnancy population." Taiwanese Journal of Obstetrics & Gynecology 54(2): 122-125.	Exclude as NIPT method not described. No reply from corresponding author.
59.	Livergood, M. C., et al. (2017). "Obesity and cell-free DNA "no calls": is there an optimal gestational age at time of sampling?" American Journal of Obstetrics & Gynecology 216(4): 413.e411-413.e419.	Exclude as NIPT method not described.
60.	Lo, K. K., et al. (2016). "Limited Clinical Utility of Non-invasive Prenatal Testing for Subchromosomal Abnormalities." American Journal of Human Genetics 98(1): 34-44.	Exclude as no testing for common trisomies T13, T18 or T21.
61.	Lu, R., et al. (2016). "Role of cell-free fetal DNA in the maternal plasma in the prenatal diagnosis of chromosomal abnormalities." International journal of clinical and experimental medicine 9(6): 11740-11747.	Exclude as NIPT method not described (possibly sequencing; performed by BGI Shenzhen Biotech Co., Ltd.).
62.	Mackie, F. L., et al. (2017). "Cell-free fetal DNA-based noninvasive prenatal testing of aneuploidy." Obstetrician & Gynaecologist 19(3): 211-218.	Exclude as non-systematic review.
63.	Manotaya, S., et al. (2016). "Clinical experience from Thailand: noninvasive prenatal testing as screening tests for trisomies 21, 18 and 13 in 4736 pregnancies." Prenatal Diagnosis 36(3): 224-231.	Exclude as sequencing, no microarray-based NIPT.
64.	Martinez-Payo, C., et al. (2018). "Clinical results after the implementation of cell-free fetal DNA detection in maternal plasma." Journal of Obstetrics and Gynaecology Research.	Exclude as NIPT method not described.
65.	McLennan, A., et al. (2016). "Noninvasive prenatal testing in routine clinical practice--an audit of NIPT and combined first-trimester screening in an unselected Australian population." Australian & New Zealand Journal of Obstetrics & Gynaecology 56(1): 22-28.	Harmony test was used, but unclear from publication if sequencing or microarray-based. Study period: March 2013 and August 2014. Agreed to exclude as study period before 10th November 2014 which was the date confirmed by Ariosa/Roche on which they started running their samples using the microarray platform.
66.	Meck, J. M., et al. (2015). "Noninvasive prenatal screening for aneuploidy: positive predictive values based on cytogenetic findings." American Journal of Obstetrics & Gynecology 213(2): 214.e211-215.	Exclude as no microarray-based NIPT (testing was performed by 4 different companies [Sequenom, Natera, Ariosa, Verinata]).
67.	Miltoft, C. B., et al. (2018). "Contingent first-trimester screening for aneuploidies with cell-free DNA in a Danish clinical setting." Ultrasound in Obstetrics & Gynecology 51(4): 470-479.	Agreed to exclude as Ariosa/Roche confirmed that the Harmony Prenatal Test with both sequencing and microarray approach was used for analysis (contradictory to information given in the publication and by the corresponding author of the paper).
68.	Minarik, G., et al. (2015). "Utilization of Benchtop Next Generation Sequencing Platforms Ion Torrent PGM and	Exclude as DNA sequencing, no microarray.

Reference		Reason for exclusion
	MiSeq in Noninvasive Prenatal Testing for Chromosome 21 Trisomy and Testing of Impact of In Silico and Physical Size Selection on Its Analytical Performance." PLoS ONE [Electronic Resource] 10(12): e0144811.	
69.	Mnyani, C. N., et al. (2016). "The value and role of non-invasive prenatal testing in a select South African population." South African Medical Journal 106(10): 1047-1050.	Exclude as Natera Panorama test with SNP-sequencing was used.
70.	Neufeld-Kaiser, W. A., et al. (2015). "Positive predictive value of non-invasive prenatal screening for fetal chromosome disorders using cell-free DNA in maternal serum: independent clinical experience of a tertiary referral center." BMC Medicine 13: 129.	Exclude as 92% of the NIPT tests were performed in one of the four major commercial laboratories offering testing during this timeframe. No information on methodology and no separate data for microarray-based methods.
71.	Neveling, K., et al. (2016). "Validation of two-channel sequencing-by-synthesis for noninvasive prenatal testing of fetal whole and partial chromosome aberrations." Prenatal Diagnosis 36(3): 216-223.	Exclude as DNA sequencing, no microarray.
72.	Norton, M. E., et al. (2016). "Cell-free DNA vs sequential screening for the detection of fetal chromosomal abnormalities." American Journal of Obstetrics & Gynecology 214(6): 727.e721-726.	Exclude as no NIPT performed (just modelled performance), no microarray.
73.	Oepkes, D., et al. (2016). "Trial by Dutch laboratories for evaluation of non-invasive prenatal testing. Part I-clinical impact." Prenatal Diagnosis 36(12): 1083-1090.	Exclude as MPSS technology was used, not microarray.
74.	Palomaki, G. E., et al. (2015). "Evaluating first trimester maternal serum screening combinations for Down syndrome suitable for use with reflexive secondary screening via sequencing of cell free DNA: high detection with low rates of invasive procedures." Prenatal Diagnosis 35(8): 789-796.	Exclude as no NIPT performed.
75.	Palomaki, G. E., et al. (2015). "Circulating cell free DNA testing: are some test failures informative?" Prenatal Diagnosis 35(3): 289-293.	Exclude as DNA sequencing not microarray based NIPT.
76.	Palomaki, G. E., et al. (2017). "The clinical utility of DNA-based screening for fetal aneuploidy by primary obstetrical care providers in the general pregnancy population." Genetics in Medicine 19(7): 778-786.	Exclude as SNP-based NIPT used (Natera), no microarray.
77.	Pantiukh, K. S., et al. (2016). "Report on noninvasive prenatal testing: Classical and alternative approaches [version 1; referees: 2 approved]." F1000Research 5 (no pagination)(722).	Exclude as whole-genome low coverage sequencing with GC correction, no microarray-based NIPT.
78.	Papageorghiou, A. T., et al. (2016). "Clinical evaluation of the IONA test: a non-invasive prenatal screening test for trisomies 21, 18 and 13." Ultrasound in Obstetrics & Gynecology 47(2): 188-193.	Exclude as Ion Proton sequencing platform was used, not microarray.
79.	Persico, N., et al. (2016). "Cell-free DNA testing in the maternal blood in high-risk pregnancies after first-trimester combined screening." Prenatal Diagnosis 36(3): 232-236.	Exclude as SNP sequencing was used (Natera), not microarray.
80.	Pertile, M. D., et al. (2017). "Rare autosomal trisomies, revealed by maternal plasma DNA sequencing, suggest increased risk of feto-placental disease." Science Translational Medicine 9(405): 30.	Exclude as whole genome sequencing, not microarray.
81.	Pescia, G., et al. (2017). "Cell-free DNA testing of an extended range of chromosomal anomalies: clinical	Exclude as analysis by shotgun sequencing on

Reference		Reason for exclusion
	experience with 6,388 consecutive cases." Genetics in Medicine 19(2): 169-175.	Illumina sequencers, not microarray-based NIPT.
82.	Petersen, A. K., et al. (2017). "Positive predictive value estimates for cell-free noninvasive prenatal screening from data of a large referral genetic diagnostic laboratory." American Journal of Obstetrics & Gynecology 217(6): 691.e691-691.e696.	Exclude as NIPT performed by a variety of commercial laboratories including Ariosa Diagnostics, BGI, Natera, Sequenom, and Illumina, according to their specific methodologies. No separate data for microarray, if used.
83.	Poon, L. C., et al. (2016). "IONA test for first-trimester detection of trisomies 21, 18 and 13." Ultrasound in Obstetrics & Gynecology 47(2): 184-187.	Exclude as NIPT using Ion Proton™sequencing platform, not microarray-based.
84.	Qi, G., et al. (2016). "Noninvasive prenatal testing in routine clinical practice for a high-risk population: Experience from a center." Medicine 95(41): e5126.	Exclude as NIPT sequencing analysis, not microarray-based NIPT.
85.	Qian, Y. Q., et al. (2018). "Detection of fetal subchromosomal aberration with cell-free DNA screening led to diagnosis of parental translocation: Review of 11344 consecutive cases in a university hospital." European Journal of Medical Genetics.	Exclude as NIPT sequencing analysis, not microarray-based NIPT
86.	Qiang, R., et al. (2017). "Detection of trisomies 13, 18 and 21 using non-invasive prenatal testing." Experimental and Therapeutic Medicine 13(5): 2304-2310.	Exclude as NIPT sequencing analysis, not microarray-based NIPT.
87.	Radoi, V. E., et al. (2015). "Cell free fetal DNA testing in maternal blood of Romanian pregnant women." Iranian Journal of Reproductive Medicine 13(10): 623-626.	Exclude as Panorama test (sequencing of SNPs), not microarray-based NIPT.
88.	Rao, R. R., et al. (2016). "The value of the first trimester ultrasound in the era of cell free DNA screening." Prenatal Diagnosis 36(13): 1192-1198.	Exclude as NIPT was performed by Sequenom (Maternity 21), Verinata (Verify), Natera (Panomara), and Ariosa (Harmony); no separate data for microarray (if used).
89.	Revello, R., et al. (2016). "Screening for trisomies by cell-free DNA testing of maternal blood: consequences of a failed result." Ultrasound in Obstetrics & Gynecology 47(6): 698-704.	Harmony Prenatal Test was used; unclear from publication if with sequencing or microarray technology. Study period: October 2012 and August 2015. Exclude as Ariosa/Roche confirmed that the Harmony Prenatal Test with both sequencing and microarray approach was used for analysis.
90.	Ryan, A., et al. (2016). "Validation of an Enhanced Version of a Single-Nucleotide Polymorphism-Based Noninvasive Prenatal Test for Detection of Fetal Aneuploidies." Fetal Diagnosis & Therapy 40(3): 219-223.	Exclude as sequencing of SNPs was used, not microarray-based NIPT.
91.	Saadati, N., et al. (2016). "Determining the role of mother race in neonatal outcome of trisomies 21, 18 and 13 using cell free DNA analysis." International Journal of Medical Research & Health Sciences 5(12): 365-369.	Exclude as no information on NIPT methodology.

Reference		Reason for exclusion
92.	Samura, O., et al. (2017). "Current status of non-invasive prenatal testing in Japan." Journal of Obstetrics & Gynaecology Research 43(8): 1245-1255.	Exclude as no separate test performance/failure data for microarray technology reported [94.7% of samples were sent to Sequenom and 5.3% were sent to four companies (Illumina; Ariosa Diagnostics; Labcorp; and Natera)].
93.	Santamaria, R., et al. (2018). "A National Referral Laboratory's Experience with the Implementation of SNP-Based Non-invasive Prenatal Screening for Fetal Aneuploidy and Select Microdeletion Syndromes." Journal of Fetal Medicine 5(1): 7-12.	Exclude as sequencing of SNPs, not microarray-based NIPT.
94.	Sarno, L., et al. (2016). "Prospective first-trimester screening for trisomies by cell-free DNA testing of maternal blood in twin pregnancy." Ultrasound in Obstetrics & Gynecology 47(6): 705-711.	Publication states that the Harmony Prenatal Test based on chromosome-selective sequencing (DANSR) was used. Study period: October 2012 and August 2015 (as Revello et al. 2016). Exclude as Ariosa/Roche stated that both approaches (NGS and microarray) were used in unclear proportions to analyse the study samples.
95.	Sbu (2015) Non-invasive prenatal test for Down's syndrome (Structured abstract). Health Technology Assessment Database	No full text available; possibly HTA report.
96.	Scott, F. P., et al. (2018). "Factors affecting cell-free DNA fetal fraction and the consequences for test accuracy." Journal of Maternal-Fetal & Neonatal Medicine 31(14): 1865-1872.	Harmony Prenatal test was used, but unclear from publication if microarray was used for DNA quantification. Mention "sequencing bias" on page 1871, so probably targeted sequencing-based testing. Study period: March 2013 and August 2014. Agreed to exclude as study period before 10th November 2014 which was the date confirmed by Ariosa/Roche on which they started running their samples using the microarray platform.
97.	Seyedoshohadaei, F., et al. (2017). "Evaluating the association between first trimester screening tests and adverse perinatal outcomes." Journal of Research in Medical and Dental Science 5(6): 14-19.	Exclude as NIPT methodology and test performance/failures not reported.
98.	Shani, H., et al. (2016). "Chromosomal abnormalities not currently detected by cell-free fetal DNA: a retrospective analysis at a single center." American Journal of Obstetrics & Gynecology 214(6): 729.e721-729.e711.	Exclude as no NIPT performed.
99.	Shi, W. L., et al. (2016). "Non-invasive prenatal testing (NIPT) detected chromosome aneuploidies and beyond in a clinical setting." International journal of clinical and experimental medicine 9(9): 18250-18254.	Exclude as sequencing-based NIPT, not microarray.

Reference		Reason for exclusion
100.	Snyder, H. L., et al. (2016). "Follow-up of multiple aneuploidies and single monosomies detected by noninvasive prenatal testing: implications for management and counseling." Prenatal Diagnosis 36(3): 203-209.	Exclude as Verifi test with MPSS was used; no microarray.
101.	Srebniak, M. I., et al. (2017). "The influence of SNP-based chromosomal microarray and NIPT on the diagnostic yield in 10,000 fetuses with and without fetal ultrasound anomalies." Human Mutation 38(7): 880-888.	Exclude as whole-genome sequencing was used, no microarray-based NIPT.
102.	Strah, D., et al. (2015). "Non-invasive prenatal cell-free fetal DNA testing for down syndrome and other chromosomal abnormalities." Zdravniski Vestnik 84(11): 727-733.	Exclude as NIPT methodology not reported (Samples were analysed at BGI Diagnostic Laboratories), most likely by sequencing-based NIPT.
103.	Strom, C. M., et al. (2017). "Improving the Positive Predictive Value of Non-Invasive Prenatal Screening (NIPS)." PLoS ONE [Electronic Resource] 12(3): e0167130.	Exclude as MPSS-based NIPT, not microarray.
104.	Sun, K., et al. (2017). "COFFEE: control-free noninvasive fetal chromosomal examination using maternal plasma DNA." Prenatal Diagnosis 37(4): 336-340.	Exclude as sequencing-based NIPT, not microarray.
105.	Suo, F., et al. (2018). "Non-invasive prenatal testing in detecting sex chromosome aneuploidy: A large-scale study in Xuzhou area of China." Clinica Chimica Acta 481: 139-141.	Exclude as sequencing-based NIPT, not microarray.
106.	Suzumori, N., et al. (2016). "Fetal cell-free DNA fraction in maternal plasma is affected by fetal trisomy." Journal of Human Genetics 61(7): 647-652.	Exclude as MPS-based NIPT, not microarray.
107.	Tan, Y., et al. (2016). "Noninvasive prenatal testing (NIPT) in twin pregnancies with treatment of assisted reproductive techniques (ART) in a single center." Prenatal Diagnosis 36(7): 672-679.	Exclude as MPS-based NIPT, not microarray-based.
108.	Taneja, P. A., et al. (2017). "Fetal aneuploidy screening with cell-free DNA in late gestation." Journal of Maternal-Fetal & Neonatal Medicine 30(3): 338-342.	Exclude as verifi Prenatal Test was used which analyses cfDNA using massively parallel next-generation whole-genome sequencing.
109.	Taneja, P. A., et al. (2016). "Noninvasive prenatal testing in the general obstetric population: clinical performance and counseling considerations in over 85000 cases." Prenatal Diagnosis 36(3): 237-243.	Exclude as verifi Prenatal Test was used which analyses cfDNA using massively parallel next-generation whole-genome sequencing.
110.	Tynan, J. A., et al. (2016). "Application of risk score analysis to low-coverage whole genome sequencing data for the noninvasive detection of trisomy 21, trisomy 18, and trisomy 13." Prenatal Diagnosis 36(1): 56-62.	Exclude as whole genome MPS based assay (VisibiliT™), not microarray.
111.	Valderramos, S. G., et al. (2016). "Cell-free DNA screening in clinical practice: abnormal autosomal aneuploidy and microdeletion results." American Journal of Obstetrics & Gynecology 215(5): 626.e621-626.e610.	Exclude as 4 commercially available laboratories were used for NIPT; no information on method used.
112.	Van Opstal, D., et al. (2018). "Origin and clinical relevance of chromosomal aberrations other than the common trisomies detected by genome-wide NIPS: Results of the TRIDENT study." Genetics in Medicine 20(5): 480-485.	Exclude as MPSS technology was used, not microarray.
113.	Verma, I. C., et al. (2018). "Single Nucleotide Polymorphism-Based Noninvasive Prenatal Testing:	Exclude as SNP-based (Natera Inc) methodology used, not microarray.

Reference		Reason for exclusion
	Experience in India." Journal of Obstetrics and Gynecology of India: 1-9.	
114.	Vicic, A., et al. (2017). "Prenatal diagnosis of Down syndrome: A 13-year retrospective study." Taiwanese Journal of Obstetrics and Gynecology 56(6): 731-735.	No NIPT performance or test failure data reported.
115.	Wang, L., et al. (2015). "Maternal mosaicism of sex chromosome causes discordant sex chromosomal aneuploidies associated with noninvasive prenatal testing." Taiwanese Journal of Obstetrics & Gynecology 54(5): 527-531.	Exclude as whole genome sequencing was used.
116.	Wang, Y. J., et al. (2017). "PLAC4 mRNA SNP in non-invasive prenatal testing of Down syndrome." International Journal of Clinical and Experimental Pathology 10(7): 7962-7967.	Exclude as PLAC4 mRNA was measured using quantitative reverse transcription-PCR; not cfDNA, not microarray.
117.	Wax, J. R., et al. (2015). "Noninvasive prenatal testing: impact on genetic counseling, invasive prenatal diagnosis, and trisomy 21 detection." Journal of Clinical Ultrasound 43(1): 1-6.	Exclude as NIPT by MPSS was used; no microarray.
118.	Williams, J., 3rd, et al. (2015). "Utilization of noninvasive prenatal testing: impact on referrals for diagnostic testing." American Journal of Obstetrics & Gynecology 213(1): 102.e101-106.	Exclude as no information on NIPT methodology and no test performance/test failure data reported.
119.	Xi, Y., et al. (2017). "Noninvasive Prenatal Detection of Trisomy 21 by Targeted Semiconductor Sequencing: A Technical Feasibility Study." Fetal Diagnosis and Therapy 42(4): 302-310.	Exclude as targeted semiconductor sequencing, not microarray-based NIPT.
120.	Xie, M. J., et al. (2018). "Noninvasive Prenatal Testing of Rare Autosomal Aneuploidies by Semiconductor Sequencing." DNA & Cell Biology 37(3): 174-181.	Exclude as semiconductor sequencing, not microarray-based NIPT.
121.	Xu, C., et al. (2017). "Noninvasive Prenatal Screening of Fetal Aneuploidy without Massively Parallel Sequencing." Clinical Chemistry 63(4): 861-869.	Exclude as high-throughput ligation-dependent probe amplification (HLPA) assay (multiplex PCR), not microarray-based NIPT.
122.	Xu, X. P., et al. (2016). "A Method to Quantify Cell-Free Fetal DNA Fraction in Maternal Plasma Using Next Generation Sequencing: Its Application in Non-Invasive Prenatal Chromosomal Aneuploidy Detection." PLoS ONE [Electronic Resource] 11(1): e0146997.	Exclude as NIPT on Ion Proton, a semiconductor sequencing platform, was used; not microarray.
123.	Yamada, T., et al. (2018). "Maternal age-specific risk for trisomy 21 based on the clinical performance of NIPT and empirically derived NIPT age-specific positive and negative predictive values in Japan." Journal of Human Genetics: 1-5.	Exclude as NIPT based on massively parallel sequencing: MaterniT21 Plus® and GeneTech NIP; no microarray.
124.	Yang, S. F., et al. (2018). "Diagnostic differences between patients opting for non-invasive prenatal testing and patients having traditional prenatal diagnosis." International Journal of Clinical and Experimental Pathology 11(5): 2831-2838.	Exclude as paper references the NIFTY test as published in Jiang 2012 which is based on MPSS.
125.	Yared, E., et al. (2016). "Obesity increases the risk of failure of noninvasive prenatal screening regardless of gestational age." American Journal of Obstetrics & Gynecology 215(3): 370.e371-376.	Exclude as NIPT based on sequencing of SNPs (Panorama test) was used, not microarray.
126.	Yaron, Y., et al. (2016). "Current controversies in prenatal diagnosis 2: for those women screened by NIPT using cell free DNA, maternal serum markers are obsolete." Prenatal Diagnosis 36(13): 1167-1171.	Exclude as no primary research article.

Reference		Reason for exclusion
127.	Yaron, Y., et al. (2017). "Current controversies in prenatal diagnosis 2: For those women screened by NIPT using cell-free DNA, maternal serum markers are obsolete." Obstetrical and Gynecological Survey 72(4): 216-217.	Exclude as note.
128.	Yu, B., et al. (2018). "Clinical evaluation of NIPS for women at advanced maternal age: a multicenter retrospective study." Journal of Maternal-Fetal and Neonatal Medicine: 1-6.	Exclude as NIPT based on sequencing, not microarray.
129.	Yu, B., et al. (2017). "Overall evaluation of the clinical value of prenatal screening for fetal-free DNA in maternal blood." Medicine 96(27): e7114.	Exclude as NIPT based on sequencing, not microarray.
130.	Zhang, H., et al. (2015). "Statistical Approach to Decreasing the Error Rate of Noninvasive Prenatal Aneuploid Detection caused by Maternal Copy Number Variation." Scientific Reports 5: 16106.	Exclude as shotgun MPS-based NIPT, not microarray.
131.	Zhang, J. and B. Zhang (2016). "Second-generation non-invasive high-throughput DNA sequencing technology in the screening of down's syndrome in advanced maternal age women." Biomedical Reports 4(6): 715-718.	Exclude as sequencing, not microarray technology.
132.	Zhang, L., et al. (2017). "Count-based size-correction analysis of maternal plasma DNA for improved noninvasive prenatal detection of fetal trisomies 13, 18, and 21." American Journal of Translational Research 9(7): 3469-3473.	Exclude as massively parallel DNA sequencing with an Ion Proton™ Sequencer, not microarray-based NIPT.
133.	Zhou, X., et al. (2017). "Contribution of maternal copy number variations to false-positive fetal trisomies detected by noninvasive prenatal testing." Prenatal Diagnosis 37(4): 318-322.	Exclude as massively parallel sequencing-based NIPT, not microarray.

Appendix 4. Information about author contacts and replies.

We contacted the corresponding authors from eight original articles that reported the use of the Harmony Prenatal Test from Ariosa/Roche for fetal trisomy 21, 18 and/or 13 detection and that were not clear about the test version used (NGS or microarray).
We also contacted the corresponding authors from two original articles that stated the use of the microarray-based test version of the Harmony Prenatal Test from Ariosa/Roche to confirm that it was used for the analyses of all samples.
We used the contact details as provided in the original publications.

The information received from the 10 contacted corresponding authors was as follows:

- Provided information was contradictory to information that we later received from Ariosa/Roche (n=1);
- Provided information 'to the best of my knowledge' was contradictory to information that we later received from Ariosa/Roche (n=1);
- Provided information was in agreement with Ariosa/Roche data (n=2);
- Did not have the information about the test methodology used (n=3);
- 'Believed' that it was the NGS-based version but suggested to better check with the lab (n=1);
- No reply from corresponding author (n=2).

In some cases, there was disagreement between the laboratory and the authors about which testing technology was used, in these cases we assumed that the laboratory was correct.

Appendix 5. Studies using cfDNA testing with both sequencing and DNA microarray technologies (7 'mixed technology' studies published in 8 articles)

Reference	Study characteristics	Findings relevant to our review
1. Bevilacqua, E., et al. (2018). "Cell-Free DNA Analysis in Maternal Blood: Differences in Estimates between Laboratories with Different Methodologies Using a Propensity Score Approach." Fetal Diagnosis and Therapy: 1-10.	Prospective cohort study comparing two different cfDNA tests. Propensity score analysis to match patients between the 2 groups. "Harmony Prenatal Test": Department of Obstetrics and Gynecology, University Hospital Brugmann, Brussels, Belgium; January 2013 and October 2016. "Cerba test": Various French fetal medicine centers and private practitioners; November 2014 and February 2016. Singleton pregnancies with cfDNA testing performed after 10 weeks of gestational age and with known pregnancy outcomes. Included: 5,505/7,121 Harmony Prenatal Test: 2,870/2,932 Cerba test: 2,635/4,189 Significant differences between the 2 groups in maternal age, maternal weight, % smokers, % higher-chance pregnancies (17% vs 61%), and gestational age. Index tests: Harmony Prenatal Test (Ariosa Diagnostics, San Jose, CA, USA) using sequencing- as well as DNA microarray-based technologies.† "Cerba test" (Laboratoire Cerba, SaintOuen-l'Aumône, France) using genome-wide massively parallel sequencing. Reference standard: "Known pregnancy outcome".	**Test accuracy (mixed chances of fetal trisomy)** **Harmony Prenatal Test** / **Cerba Test** **T21** 41/41 (100%) detected / 93/93 (100%) detected **T18** 11/12 (91.7%) detected / 7/7 (100%) detected **T13** 5/6 (83.3%) detected / 5/5 (100%) detected **FPR** 0.1% / 0.2% 1 FP for T21 / 1 FP for T21 1 FP for T18 / 3 FP for T18 1 FP for T13 / 1 FP for T13 Test performances for the detection of the major fetal trisomies 21, 18, and 13 were comparable, mainly regarding trisomy 21. The FPR was higher with the Cerba test (0.2 vs. 0.1%). **Test failures** **Harmony Prenatal Test** / **Cerba Test** **Initial test** 46/2,811 *(1.6%)* / 20/2,530 *(0.8%)* **Repeat test** 13/41 *(31.7%)* / 2/13 *(15.4%)* **Overall no-result** 18/2,811 (0.6%) / 9/1,530 (0.4%) After matching, the data indicate a higher initial no-result rate in the Harmony group (1.30%) than in the Cerba group (0.75%; p = 0.039).
2. Bjerregaard, L., et al. (2017). "The rate of invasive testing for trisomy 21 is reduced after implementation of NIPT." Danish Medical Journal 64(4).	Before-after study without concurrent control group; Aalborg University Hospital, Denmark; Before NIPT: 1 March 2011 to 1 February 2013 After NIPT: 1 March 2013 to 1 February 2015. All singleton higher-chance pregnancies (first trimester combined test chance of T21 ≥ 1:300) Before: n=253 After: n=302 (132/302 chose cfDNA testing). Index test: Harmony Prenatal Test (Ariosa Diagnostics, San Jose, CA, USA) using sequencing- as well as DNA microarray-based technologies.† Reference standard: Pre- or postnatal karyotyping or phenotype at birth.	**Test accuracy in higher-chance pregnant women with prior FTCS ≥ 1:300** (132/302 opted to have NIPT and were included in the analysis) **T21** **TP** 4 **TN** *128* **FP** 0 **FN** 0 **Test failures** **Initial test** 1/132 (0.8%) **Repeat test** 0/1 **Overall no-result** 0/132

Reference	Study characteristics	Findings relevant to our review
3. Chan, N., et al. (2018). "Implications of failure to achieve a result from prenatal maternal serum cell-free DNA testing: a historical cohort study." BJOG: An International Journal of Obstetrics and Gynaecology 125(7): 848-855.	Historical cohort study. Private specialist, multi-site prenatal screening service (Sydney Ultrasound For Women) in Sydney, Australia. June 2013 and March 2016. Women who failed to obtain a result from cfDNA testing (n=131), no exclusions from the study. cfDNA test as first-tier test? A total of 12,033 women had cfDNA testing. Harmony Prenatal Test: n=6,375 GeneSyte Test: n=5,658 Index test: Initially: Harmony Prenatal Test (Ariosa Diagnostics, San Jose, CA, USA) using sequencing- as well as DNA microarray-based technologies.† Availability of cfDNA testing during study period led to change in provider: Genea (Sydney, Australia) for analysis by GeneSyte (based on sequencing). Reference standard: NA as only data on test failures reported.	**Test failures (prior chance for fetal trisomies NR)** **Harmony Prenatal Test** / **GeneSyte Test** **Initial test** 119/6,375 (1.9%) / 12/5,658 (0.2%) **Repeat test** 13/46 (28.3%) **Overall no-result** *86/6,375 (1.3%)* *P* < 0.0001 for initial test failure rate (binomial test).
4. Gil, M. M., et al. (2016). "Clinical implementation of routine screening for fetal trisomies in the UK NHS: cell-free DNA test contingent on results from first-trimester combined test." Ultrasound in Obstetrics & Gynecology 47(1): 45-52.	Prospective cohort study. 2 NHS hospitals in England (King's College Hospital, London, and Medway Maritime Hospital, Gillingham, Kent). October 2013 and February 2015 12,134 singleton pregnancies were offered FTCS. 11,692 with known outcome included in analysis. cfDNA testing offered to women with chance of ≥ 1 in 100 (higher-chance result) and chance between 1 in 101 and 1 in 2,500 (intermediate-chance result). 3,698/4,012 (92%) chose cfDNA testing. 449/460 (97.6%) higher-chance women chose cfDNA testing. 3,249/3,552 (91.5%) intermediate-chance women chose cfDNA testing. Index test: Harmony Prenatal Test (Ariosa Diagnostics, San Jose, CA, USA) using sequencing- as well as DNA microarray-based technologies.† Reference standard: Karyotype of chorionic villi, amniotic fluid or neonatal blood or phenotype examination at birth.	**Test accuracy in intermediate(FTCS between 1:101 and 1:2,500) and higher chance (FTCS ≥ 1:100) pregnant women** (3,633/3,698 included in analysis) **T21** / **T18** / **T13** **TP** 43 / 21 / 2 **TN** *3,588* / *3,608* / *3,625* **FP** 1 / 4 / 4 **FN** 1 / 0 / 2 **Test failures** **Initial test** 99/3,698 *(2.7%)* **Repeat test** 20/54 *(37%)* **Overall no-result** 65/3,698 *(1.8%)* 3 T18

Reference	Study characteristics	Findings relevant to our review
5. Lee, T. J., et al. (2018). "Cell-free fetal DNA testing in singleton IVF conceptions." Human Reproduction 33(4): 572-578.	Retrospective cohort study. Single private obstetric and gynaecological ultrasound clinic in Melbourne, Australia. April 2013 and November 2016. 5,625 singleton pregnancies after 10 weeks' gestation had cfDNA testing performed, consecutive sampling. cfDNA testing as primary screening test before 12 weeks' gestation or as follow-on test after high-chance first or second trimester screening result. >93% first trimester. 4,633 spontaneously conceived 992 IVF. Index test: Harmony Prenatal Test (Ariosa Diagnostics, San Jose, CA, USA) using sequencing- as well as DNA microarray-based technologies.† Reference standard: Pre- or postnatal karyotype and/or phenotype at birth.	**Test accuracy (mixed chances of fetal trisomy)** *(5,569/5,625* included in analysis) **Spontaneous** / **IVF** / **Total** **T21 PPV** 40/40 (100%) / 3/3 (100%) / *43/43 (100%)* **TP** 40 / 3 / *43* **FP** 0 / 0 / *0* **T18 PPV** 10/13 (76.9%) / 1/2 (50%) / *11/15 (73.3%)* **TP** 10 / 1 / *11* **FP** 3 / 1 / *4* **T13 PPV** 1/4 (25%) / 0/5 (0%) / *1/9 (11.1%)* TP 1 / 0 / *1* FP 3 / 5 / *8* **Test failures** **Spontaneous** / **IVF** / **Total** **Initial test** 2.2% / 5.2% / NR **Overall no-result** 0.7% / 2.4% / NR
6. Miltoft, C. B., et al. (2018). "Contingent first-trimester screening for aneuploidies with cell-free DNA in a Danish clinical setting." Ultrasound in Obstetrics & Gynecology 51(4): 470-479.	Prospective cohort study. 2 hospitals in in Copenhagen (Copenhagen University Hospitals, Rigshospitalet and Herlev and Gentofte Hospital), Denmark. August 2014 and May 2015. 6,449 women aged ≥18 years with a singleton pregnancy undergoing FTCS. 597/869 with FTCS chance for T21 of ≥1 in 1,000 had cfDNA testing. Index test: Harmony Prenatal Test (Ariosa Diagnostics, San Jose, CA, USA) using sequencing- as well as DNA microarray-based technologies.† (Information from cfDNA test provider contradictory to information received from the corresponding author. We assumed the information received from the laboratory that performed cfDNA testing would be correct.) Reference standard: Pre- or postnatal karyotypes or newborn examination.	**Test accuracy in higher-chance (FTCS ≥ 1 in 1,000) pregnant women** (581/597 included in analysis) **T21** / **T18** / **T13** **TP** 13 / 1 / 2 **TN** *567* / *580* / *579* **FP** 0 / 0 / 0 **FN** 1 / 0 / 0 **Test failures** **Initial test** 19/597 (3.2%) **2nd sample** 5/7 (71.4%) **3rd sample** 2/3 (66.7%) **Overall no-result** 16/597 *(2.7%)*

Reference	Study characteristics	Findings relevant to our review
7. Revello, R., et al. (2016). "Screening for trisomies by cell-free DNA testing of maternal blood: consequences of a failed result." Ultrasound in Obstetrics & Gynecology 47(6): 698-704. Sarno, L., et al. (2016). "Prospective first-trimester screening for trisomies by cell-free DNA testing of maternal blood in twin pregnancy." Ultrasound in Obstetrics & Gynecology 47(6): 705-711.	Prospective cohort study; 2 National Health Service (NHS) hospitals in England (King's College Hospital, London, and Medway Maritime Hospital, Gillingham, Kent); 1 private clinic (Fetal Medicine Centre in London). October 2002 to August 2015. 10,963 singleton and 467 twin pregnancies had cfDNA testing and FTCS at 10-14 weeks' gestation. 10,698/10,963 singleton and 438/467 twin pregnancies with pregnancy outcome and excluding chromosomal abnormalities other than T21, T18, and T13 included for further analysis. General obstetric population, 100% first trimester. Index test: Harmony Prenatal Test (Ariosa Diagnostics, San Jose, CA, USA) using sequencing- as well as DNA microarray-based technologies.† Reference standard: Pre- or postnatal karyotypes or newborn examination.	**Test accuracy (general obstetric population)** (10,530/10,698 singleton and 417/438 twin pregnancies included in analyses) **Detection rate** **T21**: 156/158 (98.7%) singleton; 8/8 (100%) twin; *164/166 (98.8%)* total **T18**: 41/46 (89.1%) singleton; 3/4 (75%) twin; *44/50 (88.0%)* total **T13**: 8/15 (53.3%) singleton; 0/1 (0%) twin; *8/16 (50%)* total **FPR**: 23/10,311 (0.22%) singleton; 1/404 (0.25%) twin; *24/10,715 (0.22%)* total **Test failures** **Initial test**: 316/10,698 (*3.0 %*) singleton; 41/438 (9.4%) twin; *357/11,136 (3.2%)* total **Repeat test**: *87/235 (37.0%)* singleton; *19/39 (48.7%)* twin; *106/274 (38.7%)* total **Overall no-result**: *168/10,698 (1.6%)* singleton; *21/438 (4.8%)* twin; *189/11,136 (1.7%)* total

cfDNA, cell-free deoxyribonucleic acid; FN, false negative; FP, false positive; FPR, false positive rate = FP/(FP+TN) = 1 – specificity; FTCS, First trimester combined screening; NA, not applicable; NIPT, non-invasive prenatal testing (here: cfDNA testing); NR, not reported; PPV, positive predictive value; T13, trisomy 13; T18, trisomy 18; T21, trisomy 21; TN, true negative; TP, true positive.
† Information received via personal communication with Ariosa Diagnostics Inc. (San Jose, CA).
Numbers in italics were calculated based on information given in the paper.

Appendix 6. Modifications to the standard QUADAS-2 tool[7]

First, an additional signalling question was added on whether the study avoided taking the sample for the index test in the seven days after an invasive test, as fetal fraction may be elevated at this time boosting the performance of cfDNA testing.

Second, a signalling question was added to determine whether the threshold value was determined using an independent set of samples, and whether adjustment of the predefined threshold was avoided, since the threshold for testing positive is often expressed as number of standard deviations from the mean score for a set of normal samples, rather than as an absolute threshold.

Finally, the standard QUADAS-2 signalling question determining whether there was an appropriate interval between index test and reference standard was removed, as timing of an invasive test would not affect accuracy.

Timing of the cfDNA test is important as fetal fraction and therefore accuracy of cfDNA testing increases throughout pregnancy. This was included under applicability of findings rather than as a source of bias.

We also added an additional domain on the role of the sponsor to the QUADAS-2 tool. Studies were classed as high risk of bias in this domain if they clearly declared involvement of a sponsor in the design or conduct of the study or publication, the majority of authors were employees or shareholders of companies offering cfDNA testing or cytogenetic tests and/or other conflicts of interest (i.e. patents, stock or stock options) were declared.

Appendix 7. Tailored QUADAS-2 tool[7] and guidance notes

Domain 1: Patient selection

As a proportion of studies used a case-control design, the selection of study participants is of concern. This includes exclusion of hard to diagnose cases including twin pregnancies, pregnancies featuring mosaicism or translocations and homozygous foetuses in the approaches based on SNP markers.

A. Risk of bias

Guidance:

Was a consecutive or random sample of patients enrolled?

This question should only be answered with 'yes' if the study clearly states that pregnancies (rather than samples) were recruited consecutively or randomly.

Was a case-control design avoided?

For the head to head comparison question we would ideally hope for randomization to NIPT and combined test or at least a screening observational study where all participants received both tests.

For the NIPT performance question we would at least expect a prospective cohort design. Therefore, if the study is a case-control study this question should be answered with No.

Did the study avoid inappropriate exclusions?

If the study excludes >10% of participants with or without specifying reasons, the exclusions should be considered as inappropriate. This cut-off has been determined pragmatically.

B. Concerns regarding applicability

Guidance:

As the research question aims to address NIPT test performance in the first trimester and in comparison with the first trimester combined test, applicability should be regarded low if <80% of women were recruited in the first trimester.

A screening and diagnostic context should be considered separately. Low risk women without prior tests should be considered for the screening context, while high risk women should be considered for the diagnostic context (this includes add-on

and triage). Both scenarios match the different research questions but the study results will be applicable only to one of the two different contexts.

The setting where samples are taken is unlikely to have an effect on the spectrum of patients. However, the setting of the study might have an impact on the applicability of the study results to general practice in terms of feasibility, if the equipment or standards of the study setting are unlikely to be met by the routine laboratory carrying out the tests in clinical practice. Some of the technologies used in the studies might not be feasible to be carried out in routine laboratories. It needs to be decided how applicable the results of these studies are to routine practice but also whether the index test is likely to be carried out in routine laboratories or in a few specialised centers. In the UK foetal testing for sex-linked disorders and RHD genotyping is carried out in a small number of specialised centres.

Domain 2: Index test

The main sources of bias introduced by conducting and interpreting the index test are blinding and defining the threshold. Furthermore, concentrating on pregnancies with increased foetal material will bias the results, therefore, sampling should be carried out before or 7 days after invasive procedures, to avoid testing when foetal DNA levels are increased due to the invasive procedure.{Lo, #3901} If the reference standard is carried out before the index test (e.g. in case control studies) it is important to blind personnel to the karyotype results of the foetuses.

The QUADAS 2 tool requires a threshold to be pre-specified in the methods in order to avoid adjustment of the threshold according to the test outcome. However, the testing strategies considered in this review present a further level of concern. While an explicit threshold can be reported by studies (e.g. z- score>3 SD), the value of the threshold is determined by the study using either an independent set of samples or the study controls. The study threshold is therefore study specific and is dependent on the participants sampled and/or the study protocol used. This was demonstrated by one study that needed to adjust a pre-specified threshold value that a previous study had determined.{Palomaki, 2011 #3407} Since the population mean and standard deviation are not known, studies will have to determine their own threshold values. This review will, therefore, consider independent samples of participants to determine the threshold value as aiming to reduce bias.

A. Risk of bias

Were the index test results interpreted without knowledge of the results of the reference standard?

Due to the sequence of the tests, the studies need to report blinding clearly in order to answer this question with ‘yes’. Blinding can also take place by carrying out tests at different locations.

Was the sample for the index test taken before the invasive test or 7 days after invasive testing?

If the answer to this question is ‘no’, the risk of bias should be considered as ‘high’, since the accuracy of the index test will be affected by the increased amount of foetal material in the maternal circulation following invasive procedures. Lo et al. (1999) showed that testing before and 7 days after amniocentesis did not result in different

DNA levels due to rapid clearance of fetal DNA from maternal blood.{Lo, 1999 #3901}

Was a threshold explicitly pre-specified?
For this question to be answered with 'yes' the study needs to mention what kind of threshold was to be used (e.g. z-score>3SD, mean±1.96SD) and clearly state that it was specified before the start of the study.
Was the threshold value determined using an independent set of samples?
If the study used a sample of euploid controls to define an interval/threshold, the question should be answered with 'no' and the risk of bias is 'high'. A threshold determined in this way is unlikely to be robust and would lead to poorer results in an independent sample.

Studies with blinding to reference standard, blood sampling prior invasive testing, but insufficient information on the threshold used, can be classified as low-risk of bias when a commercially available non-invasive prenatal test was used.

B. Concerns about applicability
Concerns about applicability should be classified as 'high' if the index test included paternal genetic samples for all NIPT analyses.
If the study uses different screening tests to the first trimester combined test in >80%, the applicability of studies comparing NIPT to the first trimester combined test should be classed as 'high' concern about the applicability.

Domain 3: Reference standard

Due to the nature of the reference standards there is little concern about bias introduced by the choice of reference standard. We accepted prenatal or postnatal karyotyping or phenotypic newborn assessment as appropriate reference standard. They all display a detection rate of over 99% and are routine procedures in prenatal diagnosis {Dick, #3897}. If the index test is carried out before the reference standard, blinding to the results of the index tests is important.

A. Risk of bias

Is the reference standard likely to correctly classify the target condition?

Amniocentesis and CVS achieve a sensitivity and specificity of close to 100%{Dick, #3897}. Several attempts to retrieve the sample might be necessary but diagnosis is very accurate. For studies that used the stated reference standards this question should be answered with 'yes'.

Were the reference standard results interpreted without knowledge of the results of the index test?

This question should be answered with 'yes' if the routine reference standards are carried out at a different location to the index test or if the samples for the index test were stored and the index test carried out after the reference standard. However, if the question is answered with 'unclear', the risk of bias can still be regarded as low, since the laboratories carrying out the reference standards as routine tests, are unlikely to be influenced by the index test.

B. Concerns about applicability

The concern of applicability of the reference standard will be low if one of the pre-defined reference standards was used in the studies.

Domain 4: Flow and Timing

Since foetal trisomies are not progressive conditions, time intervals do not affect the performance of NIPT tests. Furthermore, all reference test have close to 100% accuracy, therefore verification bias is of little concern in studies where low risk women do not receive an invasive test but are followed up till birth. However, the exclusion of difficult to test patients and the exclusion of samples from the analysis are of great concern. These include exclusion from the study, inconclusive / intermediate results, homozygotes not testable in SNP studies, test failures and uninterpretable results.

A. Risk of bias

Did all patients receive a reference standard?

This question can be answer with 'yes' if the participants are recruited on the basis of their karyotype results.

Did all patients receive the same reference standard?

Even if this question is answered with 'no', the risk of bias can be considered as being low as long as all participants received a reference standard because all included reference standards have equally high accuracy.

Were all patients included in the analysis?

If samples were excluded due to sample issues that can be resolved by re-sampling, the risk of bias can be considered as low even if it is answered with 'no'.

However, if samples were excluded because they did not pass quality controls (e.g. amount of DNA), the risk of bias is high because this might include early pregnancies or intermediate risk pregnancies where foetal DNA levels are low.

If inconclusive or intermediate results are not included the question should be answered with 'no' and the risk of bias considered high.

Domain 5: Role of sponsor

Studies sponsored by companies are likely to be biased if the company has influence on the study design, conduct, interpretation of results and decision to publish.

A. Risk of bias

Did the funding source/sponsor play no role in design of study, interpretation of results and publication?

The risk of bias regarding the role of sponsor should be considered as' high' if studies were funded by profit-making companies and involvement of the sponsor in the design or conduct of the study or publication was stated and/or if the majority of authors or main authors were employees or shareholders of companies offering NIPT or cytogenetic tests and/or other conflicts of interest (i.e. patents, stock or stock options) were declared.

To answer this question with 'yes', the study needs to clearly state that sponsors played no role.

References

1. Lo YM, Lau TK, Zhang J, Leung TN, Chang AM, Hjelm NM, et al. Increased fetal DNA concentrations in the plasma of pregnant women carrying fetuses with trisomy 21. Clin Chem 1999;45(10):1747-51.

2. Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. Genetics in medicine : official journal of the American College of Medical Genetics 2011;13(11):913-20.

3. Dick P. Periodic health examination, 1996 update: 1. Prenatal screening for and diagnosis of Down syndrome. Canadian Task Force on the Periodic Health Examination. Canadian Medical Association Journal 1996;154(4):465-79.

Appendix 8. Study level data

Full citation	**Gil, M. M., et al. (2017). "Screening for trisomies 21 and 18 in a Spanish public hospital: from the combined test to the cell-free DNA test." Journal of Maternal-Fetal & Neonatal Medicine 30(20): 2476-2482.**
Study characteristics	
Study design / Setting / Study period	Uncontrolled before-after study. 1 centre in Spain (Torrejon University Hospital in Madrid). Before NIPT: November 2011 – December 2014; After NIPT: January 2015 – January 2016.
Population	6,011 women with singleton pregnancies attending Torrejon University Hospital in Madrid, Spain, from 11/2011-01/2016 at 11-13 weeks for first-trimester combined screening (FTCS). After NIPT introduction: 1,589 women with singleton pregnancies screened from 01/2015-01/2016. All 1st trimester (11-13 weeks' gestation for first trimester combined screening [FTCS], ~12-14 weeks for cfDNA testing). cfDNA testing offered as follow-on test to women with FTCS result > 1 in 250 without ultrasound abnormalities (nuchal translucency thickness <3.5 mm and no fetal defects). 54/72 chose cfDNA testing and were included in the analyses.
Index test / Comparator / Reference standard	Index test: Harmony Prenatal Test (Ariosa Diagnostics, Inc., San Jose, CA) performed by Ariosa Diagnostics, Inc., San Jose, CA. DANSR product quantitation method unclear from the paper; Ariosa/Roche confirmed use of DNA microarrays.† FORTE risk score ≥ 1%: high risk. Reference standard: Pregnancy outcome was ascertained at least two months after the expected due date to optimise accuracy by three methods: firstly, prenatal or postnatal karyotyping; secondly, neonatal examination and all paediatrics medical records available for the baby from Madrid region database; and thirdly, by contacting the patients' general practitioners when the previous sources were insufficient or unavailable.
Outcomes	Test accuracy of DNA microarray-based cfDNA testing (54/72 included in analysis) (cfDNA testing as follow-on test). Test failure rate (54/72 included in the analyses).

Full citation	**Gil, M. M., et al. (2017). "Screening for trisomies 21 and 18 in a Spanish public hospital: from the combined test to the cell-free DNA test." Journal of Maternal-Fetal & Neonatal Medicine 30(20): 2476-2482.**
Funding source or sponsor of the study	Funding source not reported. No conflicts of interest to declare.
Information about the authors contacted	Contacted the corresponding author via email to clarify cfDNA test methodology and missing data on true negatives and false negatives.
Information about other contacts	† Contacted Ariosa Diagnostics Inc. (San Jose, CA) via email to clarify cfDNA test methodology for study samples.

Full citation	**Juneau, K., et al. (2014). "Microarray-based cell-free DNA analysis improves noninvasive prenatal testing." Fetal Diagnosis & Therapy 36(4): 282-286.**
Study characteristics	
Study design / Setting / Study period	Retrospective study of frozen maternal plasma samples (Cohort study? Study population enriched for fetal trisomies.) Head-to-head comparison of microarray-based and next generation sequencing (NGS)-based cfDNA testing. Performed in-house by Ariosa Diagnostics (San Jose, CA). Study period NR.
Population	Singleton pregnancies in women at least 18 years old. Prior chance of fetal trisomy NR. Enriched for fetal trisomies. Exclusion of samples that did not met quality control thresholds for both quantitation methods. Gestational age: Mean 14.8 weeks, SD 4.2 weeks, Range 10-34 weeks. 392/878 with appropriate reference standard included in this review's analyses. The remaining 486 samples "*were originally tested using the Harmony Prenatal Test from Ariosa Diagnostics Inc. (San Jose, Calif., USA)*…."
Index test / Comparator / Reference standard	Index test / Comparator: Analyses of stored samples. Harmony Prenatal Test (Ariosa Diagnostics, Inc., San Jose, CA) performed by Ariosa Diagnostics, Inc., San Jose, CA. DANSR products from each sample were divided and analysed by NGS on Illumina HiSeq 2500 and by custom DNA microarrays from Affymetric Inc. imaged on an Affymetrix GeneTitan MultiChannel Instrument, respectively. For both quantitation methods: FORTE risk score ≥ 1%: high risk. Reference standard: Invasive genetic testing or postnatal newborn examination followed by detailed genetic analysis, when trisomy was suspected.
Outcomes	DNA microarray-based cfDNA test accuracy (392/878 included in analysis).[†] NGS-based cfDNA test accuracy (392/878 included in analysis).[†]
Funding source or sponsor of the study	Study designed, performed, interpreted and published by employees of Ariosa Diagnostics Inc. (San Jose, CA). All 10 authors are employees of Ariosa Diagnostics Inc. (San Jose, CA).
Information about the authors contacted	Contacted the corresponding author via email to obtain test accuracy data for the subgroup of women with appropriate reference standard.
Information about other contacts	[†] Contacted Ariosa Diagnostics Inc. (San Jose, CA) via email to obtain test accuracy data for the subgroup of women with appropriate reference standard.

Full citation	**Kagan, K. O., et al. (2018). "First-trimester risk assessment based on ultrasound and cell-free DNA vs combined screening: a randomized controlled trial." Ultrasound in Obstetrics & Gynecology 51(4): 437-444.**
Study characteristics	
Study design / Setting / Study period	Randomised controlled trial (1:1); first trimester combined screening (FTCS) versus ultrasound (US) & cfDNA testing. 1 centre in Germany (Prenatal medicine department of the University of Tuebingen). October 2015 - December 2016.
Population	Women with singleton pregnancy undergoing first-trimester screening, performed at the prenatal medicine department of the University of Tuebingen, Germany, between October 2015 and December 2016 with normal ultrasound examination (nuchal translucency thickness ≤3.5mm and no fetal defects) at 11-13 weeks' gestation. All 1st trimester, low chance of fetal trisomy. 1,400 included in study: FTCS: n=699; US & cfDNA: n=701.
Index test / Comparator / Reference standard	Index test: Harmony Prenatal Test (Ariosa Diagnostics, Inc., San Jose, CA) performed by Cenata GmbH (Tuebingen, Germany). DANSR product quantitation using DNA microarrays. FORTE risk score > 1%: high risk. Comparator: FTCS at 11-13 weeks (maternal and gestational age, fetal NT thickness, and maternal levels of serum PAPP-A and free β-hCG). Combined chance result for T21 computed based on the most recent Fetal Medicine Foundation (FMF) algorithm; cutoff: 1 in 100. Reference standard: Newborn examination or genetic testing (pre- or postnatal).
Outcomes	Test accuracy of DNA microarray-based cfDNA testing (678/701 included in analyses). Test failure rate (688/701 included in analyses.)
Funding source or sponsor of the study	Roche/Ariosa Diagnostics, Inc. (San Jose, CA, USA) provided the kits for the Harmony® Prenatal Test. Cenata GmbH (Tuebingen, Germany) performed the cfDNA analysis. One author is an employee of Roche Sequencing Solutions Inc.; another author is an employee of Cenata GmbH.
Information about the authors contacted	Contacted the corresponding author via email to clarify cfDNA test methodology.

Full citation	**Kagan, K. O., et al. (2018). "First-trimester risk assessment based on ultrasound and cell-free DNA vs combined screening: a randomized controlled trial." Ultrasound in Obstetrics & Gynecology 51(4): 437-444.**
Information about other contacts	No further contact needed.

Full citation	**Langlois, S., et al. (2017). "Comparison of first-tier cell-free DNA screening for common aneuploidies with conventional publically funded screening." Prenatal Diagnosis 37(12): 1238-1244.**
Study characteristics	
Study design / Setting / Study period	Prospective cohort study. Substudy of PEGASUS (clinicaltrials.gov identifier NCT01925742). Head-to-head comparison of first-tier microarray-based cfDNA testing and conventional screening approaches. 3/5 centres from PEGASUS study selected for this substudy: Vancouver, Calgary, Quebec (Canada). Study period NR.
Population	Women needed to be 19 years or older, have a singleton gestation, be recruited before 14 weeks gestation, have decided to undertake the provincially funded screening test. All first trimester (10 weeks – 13 weeks 6 days); general obstetric population. 1,198 women included in study.
Index test / Comparator / Reference standard	Index test: Harmony Prenatal Test (Ariosa Diagnostics, Inc., San Jose, CA) performed by Ariosa Diagnostics, Inc., San Jose, CA. DANSR product quantitation method unclear from the paper; Ariosa/Roche confirmed use of DNA microarrays.[†] Cutoff NR. Comparator: First-trimester combined screening (FTCS): 1st trimester PAPP-A, free βhCG, and nuchal translucency thickness (Calgary centre). Vancouver and Quebec centres offered serum integrated prenatal screening (SIPS) or quadruple screening to women < 35 years, and integrated prenatal screening (IPS, first-trimester ultrasound plus SIPS) for women ≥ 35 years. Reference standard: Prenatal or postnatal cytogenetic analysis, newborn and follow-up outcome at age 6 weeks.
Outcomes	Test accuracy of DNA microarray-based cfDNA testing (1,159/1,198 women included in analysis). Test accuracy of FTCS (287/300 women from Calgary centre included in analyses.) Test failure rate (1,165/1,198 women included in analyses.)
Funding source or sponsor of the study	Genome Canada, the Canadian Institutes for Health Research, Genome Québec, Genome BC, Genome Alberta, the Québec Ministère de l'enseignement supérieur, de la recherche, de la science et de la technologie. Arms' length in-kind co-funding for this study was also provided by Roche/Ariosa Diagnostics Inc (San Jose, CA) in the form of cell-free DNA (cfDNA) testing (Harmony Prenatal Test) free of charge for the women

Full citation	**Langlois, S., et al. (2017). "Comparison of first-tier cell-free DNA screening for common aneuploidies with conventional publically funded screening." Prenatal Diagnosis 37(12): 1238-1244.**
	enrolled in the present study. Roche/Ariosa Diagnostics Inc (San Jose, CA) had no role in the design of the study, interpretation of the results, or approval of the manuscript.
Information about the authors contacted	Contacted the corresponding author via email to clarify cfDNA test methodology.
Information about other contacts	† Contacted Ariosa Diagnostics Inc. (San Jose, CA) to clarify cfDNA test methodology for study samples.

Full citation	**Stokowski, R., et al. (2015). "Clinical performance of non-invasive prenatal testing (NIPT) using targeted cell-free DNA analysis in maternal plasma with microarrays or next generation sequencing (NGS) is consistent across multiple controlled clinical studies." Prenatal Diagnosis 35(12): 1243-1246.**
Study characteristics	
Study design / Setting / Study period	Multicentre cohort study; retrospective analysis. Stored (frozen) blood samples from Sweden, UK and USA. Study performed in-house by Ariosa/Roche (San Jose, CA). Study period NR.
Population	799 pregnant women (759 singleton pregnancies, 40 twin pregnancies). Prior chance of fetal trisomies NR. Apparently enriched for fetal trisomies. Gestational age at blood sampling: Median 16 (IQR 13-19) weeks' gestation.
Index test / Comparator / Reference standard	Index test: Analysis of stored samples. Harmony Prenatal Test (Ariosa Diagnostics, Inc., San Jose, CA) based on DNA microarrays; performed by Ariosa Diagnostics, Inc. (San Jose, CA). FORTE risk score ≥ 1%: high risk. Reference standard: Diagnostic testing (amniocentesis and/or chorionic villi sampling) or newborn examination with any suspected aneuploidies at birth confirmed with karyotyping.
Outcomes	Test accuracy of microarray-based cfDNA testing (791/799 included in analysis). Test failure rate (799/799 included in analyses).
Funding source or sponsor of the study	This study was supported by Ariosa Diagnostics, Inc. (San Jose, CA). 8/11 authors are paid employees of Ariosa Diagnostics.
Information about the authors contacted	No need for further contact.
Information about other contacts	No need for further contact.

Appendix 9. Quality assessment of all included studies (n=5) using the tailored QUADAS-2 tool[7]

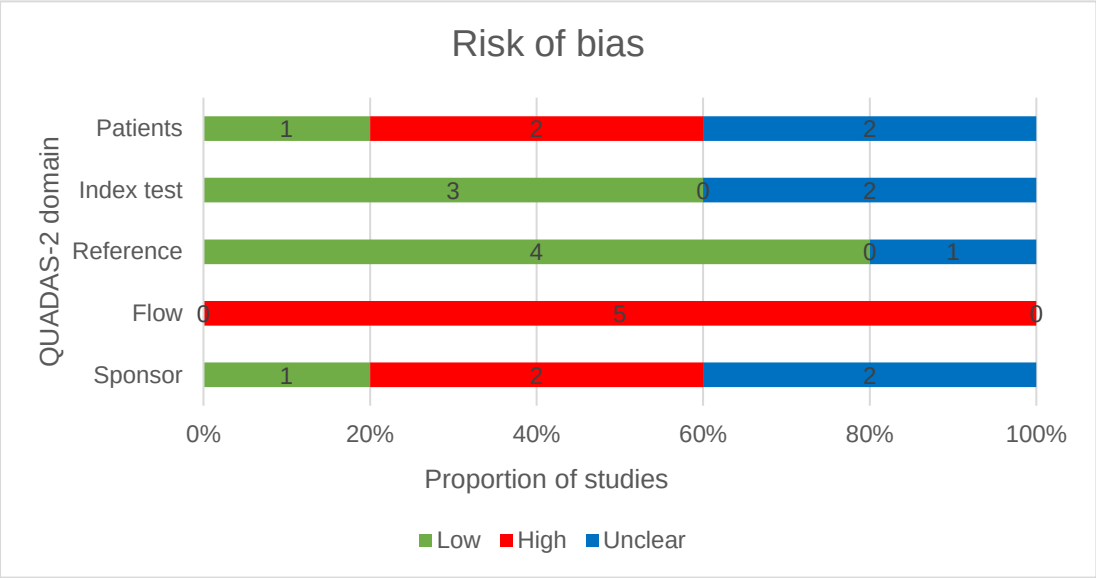
A – Individual study ratings.

Study	Risk of bias					Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Role of sponsor	Patient selection	Index test	Reference standard
Gil 2017[13]	High	Low	Unclear	High	Unclear	Low	Low†	Unclear
Juneau 2014[4]	High	Unclear	Low‡	High	High	High	Low	Low‡
Kagan 2018[10]	Low	Low	Low	High	Unclear	High	Low	Low
Langlois 2017[11]	Unclear	Low	Low	High	Low	Low	Low†	Low
Stokowski 2015[12]	Unclear	Unclear	Low	High	High	High	Low	Low

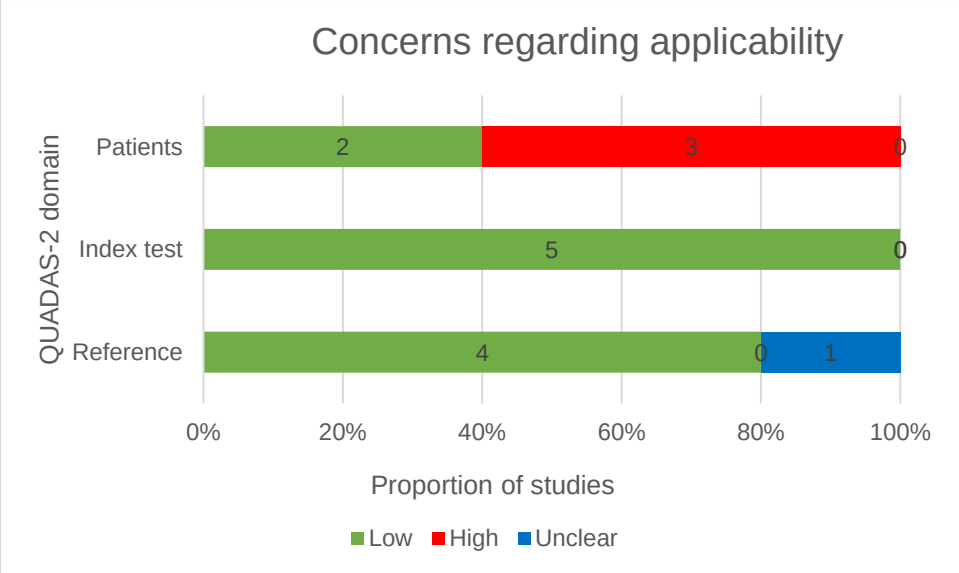
† Confirmed as DNA microarray-based cfDNA test by personal communication with Ariosa Diagnostics Inc. (San Jose, CA).

‡ Rating for subgroup of 392 samples with suitable reference standard that were included in the review's analysis.

B - Proportion of studies with low, high and unclear risk of bias for each QUADAS-2 domain.



C - Proportion of studies with low, high and unclear applicability concerns for each QUADAS-2 domain.



Appendix 10. Test accuracy outcomes in individual studies (n=5).

Reference	Fetal fraction	2x2 table					Sensitivity% (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Other	Test failures / inconclusive results / other exclusions from analysis
			TP	TN	FP	FN						
Gil 2017[13]	NR	T21	1	53	0	0	*100 (5.46-100)*	*100 (91.6-100)*	*100 (5.46-100)*	*100 (91.6-100)*	NR	0 test failures after repeat test / 0 inconclusive results / 18 women with higher-chance FTCS result did not choose cfDNA testing; 5,939 not offered cfDNA testing.
		T18	0	*54*	0	*0*	NA	*100 (91.7-100)*	NA	*100 (91.7-100)*		
		T13	0	*54*	0	*0*	NA	*100 (91.7-100)*	NA	*100 (91.7-100)*		
Juneau 2014[4] DNA microarray-based cfDNA test	NR	T21	72†	*320*†	0†	0†	*100 (93.7-100)*	*100 (98.5-100)*	*100 (93.7-100)*	*100 (98.5-100)*	NR	Test failures excluded from study / 0 inconclusive results / 486 with unclear reference standard.
		T18	13†	*379*†	0†	0†	*100 (71.7-100)*	*100 (98.7-100)*	*100 (71.7-100)*	*100 (98.7-100)*		
		T13	7†	*385*†	0†	0†	*100 (56.1-100)*	*100 (98.8-100)*	*100 (56.1-100)*	*100 (98.8-100)*		
Sequencing-based cfDNA test	NR	T21	72†	*320*†	0†	0†	*100 (93.7-100)*	*100 (98.5-100)*	*100 (93.7-100)*	*100 (98.5-100)*	NR	Test failures excluded from study / 0 inconclusive results / 486 with unclear reference standard.
		T18	13†	*379*†	0†	0†	*100 (71.7-100)*	*100 (98.7-100)*	*100 (71.7-100)*	*100 (98.7-100)*		
		T13	7†	*385*†	0†	0†	*100 (56.1-100)*	*100 (98.8-100)*	*100 (56.1-100)*	*100 (98.8-100)*		
Kagan 2018[10] cfDNA testing	Median 12.5%	T21	*0*	*678*	*0*	*0*	NA	*100 (99.3-100)*	NA	*100 (99.3-100)*	FP rate T21: 0%	10 test failures / 0 inconclusive results / 13 without reference standard.
		T18	*0*	*678*	*0*	*0*	NA	*100 (99.3-100)*	NA	*100 (99.3-100)*		
		T13	*0*	*678*	*0*	*0*	NA	*100 (99.3-100)*	NA	*100 (99.3-100)*		
FTCS	NA	T21	0	671	17	0	NA	*97.5 (96.0-98.5)*	*0 (0-22.9)*	*100 (99.3-100)*	FP rate T21: 2.5%	0 test failures / 0 inconclusive results / 11 without reference standard.

Reference	Fetal fraction	2x2 table					Sensitivity% (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Other	Test failures / inconclusive results / other exclusions from analysis
			TP	TN	FP	FN						
Langlois 2017[11]	NR	T21	6	*1,153*	0	0	*100 (51.7-100)*	*100 (99.6-100)*	*100 (51.7-100)*	*100 (99.6-100)*	FP rate T21: 0%	6 test failures after repeat test /
cfDNA testing		T18	0	*1,158*	1	0	NA	*99.9 (99.4-100)*	*0 (0-94.5)*	*100 (99.6-100)*	FP rate T18: 0.09%	0 inconclusive results / 30 without reference standard,
		T13	0	*1,158*	1	0	NA	*99.9 (99.4-100)*	*0 (0-94.5)*	*100 (99.6-100)*	FP rate T13: 0.09%	3 wrong gestational dating.
FTCS	NA	T21	5	263	19	0	*100 (46.3-100)*	*93.3 (89.5-95.8)*	*20.8 (7.9-42.7)*	*100 (98.2-100)*	NR	0 test failures / 0 inconclusive results / 33 without reference standard / 878 other standard screening test than FTCS.
Stokowski 2015[12]	Median 13.8% IQR 10.7-16.9%	T21	107	*683*	0	1	99.1 *(94.2-99.95)*	100 *(99.3-100)*	*100 (95.7-100)*	*99.85 (99.1-99.99)*	NR	8 test failures / 0 inconclusive results.
		T18	29	*761*	0	1	96.7 *(80.9-99.8)*	100 *(99.4-100)*	*100 (85.4-100)*	*99.87 (99.2-99.99)*		
		T13	12	*779*	0	0	100 *(69.9-100)*	100 *(99.4-100)*	*100 (69.9-100)*	*100 (99.4-100)*		

cfDNA, cell-free deoxyribonucleic acid; CI, confidence interval; FTCS, First trimester combined screening; FP, false positive; FP rate = FP / (FP+TN) = 1 – Specificity; FN, false negative; IQR, interquartile range (25th-75th percentiles); NA, not applicable; NR, not reported; NPV, negative predictive value; PPV, positive predictive value; TN, true negative; TP, true positive.

Note: Numbers in italics were calculated by reviewers based on information given in the paper. Confidence intervals in italics were calculated using the Wilson score interval with continuity correction.[8,9] Numbers and confidence intervals not in italics were extracted directly from the papers.

† Unpublished data received from Ariosa Diagnostics Inc. (San Jose, CA) on 12th October 2018.

Appendix 11. Initial test failure rates, reasons for failure and success of repeat tests (4 studies)

Reference / Population	Initial cfDNA test failure rate	Repeat tests successful	Causes of cfDNA test failures
Gil 2017[13] Singleton pregnancies at higher chance of fetal trisomies *12-14 weeks*	1/54 *(1.9%)*	2nd blood draw: 1/1	NR
Kagan 2018[10] Singleton pregnancies at low chance of fetal trisomies 11-13 weeks	10/688 (1.5%)	Not performed	NR
Langlois 2017[11] Singleton pregnancies, no prior testing 10-14 weeks	11/1,165 (0.9%) (95%CI 0.47-1.7%)	2nd blood draw: 5/11 (45.5%)	1st blood draw: 10/11 low fetal fraction; 1/11 unusually high variance in cfDNA count.
Stokowski 2015[12] Singletons & twins, apparently enriched for fetal trisomies Median 16 weeks (IQR 13-19 weeks).	8/799† *(1.0%)*	NR	8/8 insufficient fetal DNA
Total (4 studies)	***30/2,706 (1.1%)*** ***(95%CI 0.8% to 1.6%)***	**NA**	**NA**

cf, cell-free; CI, confidence interval; DNA, deoxyribonucleic acid; IQR, Interquartile range (25th-75th percentiles); NA, not applicable; NR, not reported.

† Unclear if the reported failure rate is after initial testing or includes repeat testing.

Numbers in italics are calculated by reviewers from information given in the paper. Confidence intervals in italics were calculated using the Wilson score interval with continuity correction.[8,9] Numbers and confidence intervals not in italics were extracted directly from the papers.

References relevant to the appendices

4. Juneau K, Bogard PE, Huang S*, et al.* Microarray-based cell-free DNA analysis improves noninvasive prenatal testing. Fetal Diagn Ther. 2014;36(4):282-286.

7. Whiting PF, Rutjes AW, Westwood ME*, et al.* QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Annals of internal medicine. 2011;155(8):529-536.

8. Wilson EB. Probable Inference, the Law of Succession, and Statistical Inference. Journal of the American Statistical Association. 1927;22(158):209-212.

9. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. Stat Med. 1998;17(8):857-872.

10. Kagan KO, Sroka F, Sonek J*, et al.* First-trimester risk assessment based on ultrasound and cell-free DNA vs combined screening: a randomized controlled trial. Ultrasound in Obstetrics & Gynecology. 2018;51(4):437-444.

11. Langlois S, Johnson JA, Audibert F*, et al.* Comparison of first-tier cell-free DNA screening for common aneuploidies with conventional publically funded screening. Prenat Diagn. 2017;37(12):1238-1244.

12. Stokowski R, Wang E, White K*, et al.* Clinical performance of non-invasive prenatal testing (NIPT) using targeted cell-free DNA analysis in maternal plasma with microarrays or next generation sequencing (NGS) is consistent across multiple controlled clinical studies. Prenat Diagn. 2015;35(12):1243-1246.

13. Gil MM, Brik M, Casanova C*, et al.* Screening for trisomies 21 and 18 in a Spanish public hospital: from the combined test to the cell-free DNA test. Journal of Maternal-Fetal & Neonatal Medicine. 2017;30(20):2476-2482.