Washington State Health Care Authority

Cell-free DNA Prenatal Screening for Chromosomal Aneuploidies

Draft evidence report

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This health technology assessment report is based on research conducted by the Center for Evidence-based Policy (Center) under contract to the Washington State Health Care Authority (HCA). This report is an independent assessment of the technology question(s) described based on accepted methodological principles. The findings and conclusions contained herein are those of the authors, who are responsible for the content. These findings and conclusions do not necessarily represent the views of the Washington HCA and thus, no statement in this report shall be construed as an official position or policy of the HCA.

The information in this assessment is intended to assist health care decision makers, clinicians, patients, and policy makers in making evidence-based decisions that may improve the quality and cost-effectiveness of health care services. Information in this report is not a substitute for sound clinical judgment. Those making decisions regarding the provision of health care services should consider this report in a manner similar to any other medical reference, integrating the information with all other pertinent information to make decisions within the context of individual patient circumstances and resource availability.

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List of Abbreviations

45,X	Turner syndrome
47,XXX	Triple X syndrome
47,XXY	Klinefelter syndrome
47,XYY	Jacob's syndrome
ACOG	American College of Obstetricians and Gynecologists
AFP	alpha-fetoprotein
AMA	advanced maternal age
ART	assisted reproductive techniques
BMI	body mass index
CCMG	Canadian College of Medical Geneticists
cfDNA	cell-free DNA
cFTS	combined first-trimester screening
CI	confidence interval
CMA	chromosomal microarray analysis
CMS	Centers for Medicare and Medicaid Services
CoE	certainty of evidence
CPT	Current Procedural Terminology
CVS	chorionic villus sampling
CY	calendar year
EED	NHS Economic Evaluation Database
FASP	Fetal Anomaly Screening Programme
FFS	fee-for-service
FN	false negative
FP	false positive
FTS	first-trimester screening
GRADE	Grading of Recommendations, Assessment, Development, and Evaluation
hCG	human chorionic gonadotropin
HGSA	Human Genetics Society of Australasia
HTA	Health Technology Assessment Program
ICD	International Statistical Classification of Diseases and Related Health Problems
ICER	incremental cost-effectiveness ratio
IPS	integrated screening with SIPS and NT
KQ	key question
IQR	inter-quartile range
IVF	in vitro fertilization
MA	maternal age
MMS	multiple-marker screening

MoM	multiples of the median
MPSS	massively parallel shotgun sequencing
MSS	maternal serum screen
NB	nasal bone
NIHR	National Institute for Health Research
NIPS	noninvasive prenatal screening
NIPT	noninvasive prenatal testing
NPV	negative predictive value
NR	not reported
NT	nuchal translucency
PAPP-A	pregnancy-associated plasma protein A
PEBB/UMP	Public Employees Benefit Board Uniform Medical Plan
PPV	positive predictive value
QUAD	second-trimester quadruple screening
QALY	quality-adjusted life year
RANZCOG	Royal Australian and New Zealand College of Obstetricians and Gynaecologists
RCT	randomized controlled trial
RR	risk ratio
SCA	sex chromosome abnormality
SD	standard deviation
SIPS	first-trimester PAPP-A, second-trimester AFP, hCG, uE3, inhibin A
SMFM	Society of Maternal and Fetal Medicine
SNP	single nucleotide polymorphism;
SOGC	Society of Obstetricians and Gynaecologists of Canada
T13	trisomy 13
T18	trisomy 18
T21	trisomy 21
TMPS	targeted massively parallel sequencing
TN	true negative
TP	true positive
uE3	unconjugated estriol 3
UN	United Nations

Executive Summary

Structured Abstract

Purpose

This report reviews the effectiveness and cost-effectiveness of cell-free DNA (cfDNA) aneuploidy screening for general obstetric populations.

Data Sources

We searched Ovid MEDLINE, Ovid MEDLINE In-Process & Other Non-Indexed Citations, and Ovid MEDLINE Epub Ahead of Print; the Cochrane Database of Systematic Reviews and the Cochrane Central Register of Controlled Trials; Scopus; the NHS Economic Evaluation Database (EED) and the National Institute for Health Research Health Technology Assessment Program (HTA) database; the U.S. National Library of Medicine clinical trials registry; relevant clinical practice guidelines; and public and private payer coverage policies. We searched for studies published between 2007 and July 2019.

Study and Guideline Selection

Using *a priori* criteria, we conducted dual independent title and abstract screening and full-text article review for English language randomized controlled trials (RCTs), observational studies, and economic evaluations of cfDNA prenatal screening. A third reviewer settled discrepancies. We also selected relevant clinical practice guidelines, using a similar process.

Data Extraction and Risk of Bias Assessment

One researcher used standardized procedures to extract data from the included studies and a second researcher checked all data entry for accuracy. We performed dual independent risk-of-bias assessment on the included studies and guidelines. A third reviewer settled discrepancies.

Data Synthesis and Analysis

We applied the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) working group system to rate the overall quality of evidence on selected measures of pregnancy outcomes and test performance.

Results

We screened a total of 2,113 records and found 1 RCT (reported in 3 publications), 9 test accuracy studies, and 8 economic studies that met our inclusion criteria. We assessed 2 studies, both economic studies, as having a low risk of bias and all others as moderate or high risk of bias.

The impact of prenatal screening using cfDNA was assessed in 10 studies. We found that cfDNA screening:

- Has a lower false-positive (FP) screening rate than conventional first-trimester aneuploidy screening (FTS) (0% vs. 2.5%; *P* value not reported) (low-quality evidence)
- Has a test failure rate ranging from 0.9% to 8.5% (very-low-quality evidence)

- Results in lower rates of invasive testing than conventional aneuploidy screening (low-to-very-low-quality evidence)

Based on the 9 studies evaluating test accuracy, we also found that cfDNA screening:

- Results in fewer or the same number of missed cases of aneuploidy as conventional screening (moderate-to-very-low quality evidence)
- Results in fewer women undergoing unnecessary testing compared with conventional aneuploidy screening (moderate quality evidence)
- Has a higher positive predictive value (PPV) than conventional aneuploidy screening (moderate-to-very-low quality evidence)

We found limited evidence on the performance of cfDNA screening for common sex chromosome abnormalities and twin pregnancies.

Universal cfDNA screening was more effective than conventional aneuploidy screening in most of the economic studies we reviewed but the results varied depending on whether cfDNA represented value for money (low quality evidence). The economic models produced similar results to the test performance studies, with cfDNA screening identifying more cases of aneuploidy and reducing invasive testing.

Clinical Practice Guidelines and Payer Policies

We found 13 eligible guidelines, 2 of which we assessed as having high methodological quality. The guidelines on the use of cfDNA screening in general obstetric populations differed between the 2 high-methodological quality guidelines. Both cited the resource impact and implementation challenges of cfDNA screening.

- The Human Genetics Society of Australasia (HGSA) and the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) agreed that there was sufficient evidence for the use of cfDNA as a primary screening test for women with singleton pregnancies. In twin pregnancies, HGSA and RANZCOG recommend that cfDNA-based screening may be offered with appropriate pre-test counselling regarding the increased test failure rate for multifetal pregnancies and a smaller evidence base compared with singleton pregnancies.
- All pregnant women in England are offered conventional screening for trisomy 21 (Down syndrome, T21), trisomy 18 (Edwards syndrome, T18), and trisomy 13 (Patau syndrome, T13) as part of the NHS Fetal Anomaly Screening Programme. cfDNA testing is not currently offered to all women.

Other guidelines generally recommended that all pregnant women be informed of aneuploidy screening options for T21, T18, and T13, including cfDNA. The guidelines also emphasized the importance of discussing the implications of testing with a professional with expertise in genetic testing and counseling.

We did not find any Medicare National or Local Coverage Determinations on cfDNA prenatal screening. Of the 3 private payers (Aetna, Cigna, and Regence), 2 covered cfDNA screening for aneuploidies in the general obstetric population. All of the private payers considered cfDNA screening to be investigational

or experimental for sex chromosome aneuploidies and 2 considered it to be investigational or experimental in multifetal pregnancies.

Conclusions

Based on the evidence reviewed in this report, universal cfDNA aneuploidy screening appears to be an accurate method of screening for the common trisomies (T21, T18, and T13) in general obstetric populations. However, universal cfDNA testing is likely to be more expensive than conventional screening depending on the exact costs of the cfDNA test used. Policy makers therefore need to consider the value of expanding cfDNA screening to all pregnant women and whether it is worth the additional associated costs. The economics studies included in this report suggest that universal cfDNA screening can be cost-effective, particularly when the lifetime costs of T21, T18, and T13 and the wider societal costs are included. There is a lack of clinical and cost-effectiveness evidence on the use of cfDNA screening for sex chromosome aneuploidies. Clinical practice guidelines generally recommend that women be informed of the range of tests that are available for prenatal screening, but recommendations regarding the most appropriate test for universal screening in the general obstetric population differ.

Background

Prenatal screening is a part of standard maternity care and includes a range of tests and evaluations to determine the health of mother and fetus. Specifically, prenatal genetic screening assesses whether a patient's fetus carries an increased risk of being affected by a genetic disorder.[1] In contrast, prenatal genetic diagnostic testing determines, as definitively as possible, whether a specific genetic disorder or condition is present in the fetus.[1] Prenatal screening for aneuploidy, usually performed during the first or second trimester of pregnancy, assesses a woman's risk that she is carrying a fetus with one of the more common fetal chromosomal aneuploidies.[1]

Technology of Interest

cfDNA screening is noninvasive prenatal testing or screening (NIPT) used to determine the risk that a fetus has certain genetic abnormalities.[2] cfDNA testing analyzes fragments of fetal DNA present in maternal blood[2] and is considered noninvasive compared with traditional testing methods such as amniocentesis or chorionic villus sampling. The cfDNA in a maternal blood sample can be screened for T21 (Down syndrome), T18 (Edwards syndrome), T13 (Patau syndrome), and abnormalities involving the number of sex chromosomes, such as Klinefelter syndrome (47,XXY) and Turner syndrome (45,X).[1]

Policy Context

cfDNA testing is used for prenatal screening for common chromosomal abnormalities. Uncertainty exists regarding the appropriateness of cfDNA screening for some populations, including those at low risk for common fetal genetic abnormalities. This topic was selected for a health technology assessment due to medium concerns about the safety and efficacy of cfDNA screening in the general obstetric population and high concerns about cost.

Methods

This review is based on final key questions (KQs) published on August 6 and updated on August 26, 2019.[3] The draft KQs were available for public comment from July 10 to July 23, 2019, and appropriate revisions were made to the KQs based on the comments.[4]

Key Questions

1. What is the evidence on the efficacy and effectiveness of using cfDNA in pregnant individuals not known to be at high risk for chromosomal abnormalities:
    a. For T21, T18, and T13, compared to active screening approaches, including standard screening with serum biomarkers and ultrasound, screening with another cfDNA screening test, question-based screening, or invasive diagnostic testing?
    b. For common sex chromosome aneuploidies, any active screening approach, screening with another cfDNA screening test, no screening, or invasive diagnostic testing?

2. What direct harms are associated with screening using cfDNA in pregnant individuals not known to be at high risk for chromosomal abnormalities:
    a. For T21, T18, and T13, compared to active screening approaches, including standard screening with serum biomarkers and ultrasound, screening with another cfDNA screening test, question-based screening, or invasive diagnostic testing?

   b. For common sex chromosome aneuploidies, any active screening approach, screening with another cfDNA screening test, no screening, or invasive diagnostic testing?

3. Do important efficacy/effectiveness outcomes or direct harms of screening for T21, T18, and T13 and for common sex chromosome aneuploidies using cfDNA vary for the mother and fetus or infant by:
   a. Maternal characteristics (e.g., age)
   b. Singleton or multifetal pregnancy
   c. Timing of screening (e.g., gestational age)

4. What are the cost-effectiveness and other economic outcomes of screening for T21, T18, and T13 and for common sex chromosome aneuploidies using cfDNA in pregnant individuals not known to be at high risk for chromosomal abnormalities?

We also included a contextual question on the benefits and harms of cfDNA screening for T21, T18, and T13 and for common sex chromosome aneuploidies in high-risk populations.

Data Sources and Searches

We searched Ovid MEDLINE, Ovid MEDLINE In-Process & Other Non-Indexed Citations, and Ovid MEDLINE Epub Ahead of Print from 2007 to July 8, 2019; the Cochrane Database of Systematic Reviews and the Cochrane Central Register of Controlled Trials from 2007 to July 8, 2019; Scopus through July 9, 2019; the EED and HTA databases from 2007 to July 9, 2019; the National Library of Medicine clinical trials registry; relevant professional society and organization clinical practice guidelines; and public and private payer coverage policies.

Study and Guideline Selection

Using *a priori* criteria, we conducted dual independent title and abstract screening and full-text article review for English language randomized controlled trials (RCTs), observational studies, and economic evaluations of prenatal screening using cfDNA. A third reviewer settled discrepancies. We also selected relevant clinical practice guidelines, using a similar process.

Data Abstraction and Quality Assessment

One researcher used standardized procedures to extract data from the included studies and a second researcher checked all data entry for accuracy. We performed dual independent risk-of-bias assessment on the included studies and guidelines. A third reviewer settled discrepancies.

Data Analysis and Synthesis

We extracted 2x2 tables for studies of test performance and calculated standard test accuracy measures (e.g., sensitivity, PPV) using the MedCalc online diagnostic calculator.[5] We also used RevMan software to graphically present test sensitivities and specificities by test and condition.[6] We applied the GRADE system to rate the overall quality of evidence on selected measures of pregnancy outcomes and test performance. Meta-analysis was not possible because we only found 1 RCT reporting pregnancy outcomes.

Results

Our searches returned a total of 2,109 records and we added an additional 4 records from other sources. Of these, 1 RCT (reported in 3 publications), 9 test accuracy studies, and 8 economic studies met our inclusion criteria. For purposes of GRADE ratings, the function measures we selected were FP rate, test failures, rates of invasive testing, and test performance (i.e., sensitivity, specificity, PPV, and negative predictive value [NPV]).

Contextual Question 1

A Cochrane review published in 2017 evaluated the diagnostic accuracy of cfDNA screening as a first-tier test in unselected populations of pregnant women undergoing aneuploidy screening or as a second-tier test in pregnant women considered to be at high risk after first-tier screening for common fetal aneuploidies.[7] The Cochrane review concluded that "non-invasive prenatal testing methods appear to be sensitive and highly specific for detection of fetal trisomies 21, 18, and 13 in high-risk populations."[7] However, the authors emphasized that invasive fetal karyotyping remains the required diagnostic approach to confirm the presence of a chromosomal abnormality prior to making irreversible decisions relative to the pregnancy outcome.[7]

Key Questions 1 and 2

The impact of prenatal cfDNA screening was assessed in 10 studies.[8-17] These 10 studies[8-17] found that screening with cfDNA:

- Has a lower FP screening rate than conventional FTS (0% vs. 2.5%; *P* value not reported) (low-quality evidence, based on 1 RCT)
- Has a test failure rate ranging from 0.9% to 8.5% (very-low-quality evidence, based on data from 1 RCT, 8 cohort studies, and 1 case-control study)
- Results in lower rates of invasive testing than conventional screening (low-quality evidence based on 1 RCT and very-low-quality evidence from 2 cohort studies)

Key Question 3

No studies compared outcomes or test performance by maternal age. Three studies found that greater maternal weight was associated with higher rates of cfDNA test failures.[12,14,15] Only 2 studies included twin pregnancies,[11,15] but direct comparisons of outcomes or test performance were not conducted for singleton and multifetal pregnancies. In 1 study, a slightly higher occurrence of test failures in low-risk women compared with the overall cohort (8.5% vs. 8.1%; $P = .86$) was attributed to a lower gestational age at testing (a median of 12.9 weeks vs. 14.3 weeks).[16] In 1 study, the prevalence of aneuploidies was lower in women with a successful cfDNA test compared with women with a failed cfDNA test (0.4% vs. 2.7%; $P < .001$).[14] The prevalence of aneuploidies in women with a low fetal fraction (i.e., the percentage of DNA in the maternal blood sample from the fetus) was 4.7%.[14]

Key Question 4

Based on the 8 included economic studies,[18-25] universal cfDNA screening was more effective than conventional screening in the majority of studies we reviewed, but the results differed on whether cfDNA represented value for money (low quality evidence). The economic models produced similar

results to the test performance studies, with cfDNA screening identifying more cases of aneuploidy and reducing the number of invasive diagnostic tests and associated procedure-related pregnancy losses.

Summary

Universal screening with cfDNA appears to be an accurate method for identifying the common trisomies (T21, T18, and T13) in general obstetric populations. However, universal cfDNA testing is likely to be more expensive than conventional screening, depending on the exact costs of the cfDNA test.

Clinical Practice Guidelines

We found 13 eligible guidelines,[1,26-37] of which 2 were assessed as having high methodological quality. Recommendations on cfDNA screening in the general obstetric population differed between the 2 guidelines and both cited the resource impacts and implementation challenges of universal cfDNA screening.

- The HGSA and RANZCOG agreed that there was sufficient evidence for the use of cfDNA screening as a primary screening test for fetal aneuploidy in women with singleton pregnancies.[30] In twin pregnancies, the guidelines recommended that cfDNA screening may be offered with appropriate pre-test counselling regarding the increased test failure rate and the lack of research data for multifetal pregnancies compared with singleton gestations. The choice of a screening test, either a combined FTS or cfDNA, depends on local resources, patient demographics, and individual patient characteristics.[30]
- All pregnant women in England are offered a combined test for common trisomies (T21, T18 and T13) as part of the NHS Fetal Anomaly Screening Programme (FASP).[30] Pregnant women at a higher risk of having a baby with 1 of these conditions are offered follow-up diagnostic tests.[30] Currently, cfDNA testing is not part of routine NHS screening programs.[30]

Other guidelines generally recommend that all pregnant women be informed of the prenatal screening options, including cfDNA, for T21, T18, and T13.[1,26-29,31,32,34-37] The guidelines also emphasize the importance of discussing the implications of testing with a professional with expertise in genetic testing and counseling.

Selected Payer Coverage Determinations

We did not identify any Medicare National or Local Coverage Determinations regarding prenatal cfDNA screening. Of the 3 private payers (Aetna, Cigna, and Regence), 2 cover cfDNA aneuploidy screening in the general obstetric population.[38-40] Aetna, Cigna, and Regence consider cfDNA screening to be investigational or experimental for sex chromosome aneuploidies and 2 of the commercial payers also consider cfDNA screening to be investigational or experimental in multifetal pregnancies.[38-40]

Ongoing Studies

We identified 1 ongoing RCT comparing universal cfDNA screening with cfDNA screening after a positive FTS.[41] The study is expected to be completed in December 2021.[41]

Conclusions

Based on the evidence reviewed in this report, universal screening with cfDNA appears to be an accurate method of screening for the common trisomies (T21, T18, and T13) in the general obstetric population. However, universal cfDNA testing is likely to be more expensive than conventional

screening, depending on the exact costs of the cfDNA test used. Policy makers therefore need to consider the value of expanding cfDNA screening to all pregnant women and whether it is worth the additional associated costs. The economics studies included in this report suggest that universal cfDNA screening can be cost-effective, particularly when the lifetime costs of trisomies T21, T18, and T18 and the wider societal costs are included. There is a lack of clinical and cost-effectiveness evidence regarding the use of cfDNA screening for sex chromosome aneuploidies. Clinical practice guidelines generally recommend that women be informed of the range of tests available for prenatal screening, but recommendations differ in terms of the most appropriate test for universal screening in the general obstetric population.

Technical Report

Background

Prenatal screening is a part of standard maternity care and includes a range of tests and evaluations to determine the health of mother and fetus. Specifically, prenatal genetic screening assesses whether a patient's fetus carries an increased risk of being affected by a genetic disorder.[1] In contrast, prenatal genetic diagnostic testing determines, as definitively as possible, whether a specific genetic disorder or condition is present in the fetus.[1] Prenatal screening for aneuploidy, usually performed during the first or second trimester of pregnancy, assesses a woman's risk that she is carrying a fetus with one of the more common fetal chromosomal aneuploidies.[1] Prenatal screening for aneuploidy is generally offered as follows:

- First-trimester screening (FTS) is typically performed between 10 and 13 weeks of gestation.[1] It includes measurements of nuchal translucency using ultrasound and of either serum free-β-human chorionic gonadotropin (hCG) or total hCG and pregnancy-associated plasma protein A (PAPP-A) analyte levels in maternal blood samples.[1] A specific risk estimate for aneuploidy is calculated using these results as well as maternal factors such as age, prior history of aneuploidy, weight, race, and number of fetuses.[1]
- Second-trimester screening, also called the quadruple marker (QUAD) screen, is typically performed between 15 and 22 weeks of gestation.[1] It includes measurements of 4 maternal serum analytes (hCG, alpha-fetoprotein, dimeric inhibin A, and unconjugated estriol) in combination with maternal factors such as age, weight, race, the presence of diabetes, and the number of fetuses to estimate the risk of aneuploidies and open fetal defects.[1]

The risk estimates obtained during the first and second trimesters can be combined with ultrasound results (combined screening) to improve the detection of fetal anomalies. The results of maternal blood screens for fetal aneuploidy represent the level of risk that a disorder might be present:

- A positive screening test for aneuploidy indicates that the fetus is at higher risk of having a disorder compared with the general population. It does not definitively diagnose a disorder.[1]
- A negative result indicates that the fetus is at lower risk of having a disorder compared with the general population. It does not definitively rule out the possibility that the fetus has a disorder.[1]

Screening for aneuploidies involves identifying chromosomal disorders caused by an extra or missing copy (aneuploidy) of a chromosome.[2] Screened disorders typically include Down syndrome (trisomy 21, caused by an extra chromosome 21), Edwards syndrome (trisomy 18, caused by an extra chromosome 18), Patau syndrome (trisomy 13, caused by an extra chromosome 13), and extra or missing copies of the X and Y chromosomes (sex chromosomes).[2] These aneuploidies display the following characteristics:

- Down syndrome (T21) is a chromosomal condition associated with intellectual disability, a characteristic facial appearance, and weak muscle tone (hypotonia) in infancy.[42] All affected individuals experience cognitive delays but their intellectual disabilities are usually mild to moderate.[42] Down syndrome (T21) occurs in about 1 in 800 newborns.[42] About 5,300 babies with Down syndrome are born in the U.S. each year and approximately 200,000 people in the U.S. currently have the condition.[42] Although women of any age can have a child with Down syndrome, the chance of having a child with this condition increases with maternal age.[42]

- Edwards syndrome (T18) is a chromosomal condition associated with abnormalities in many parts of the body.[43] Fetuses often experience slow growth (intrauterine growth restriction) and are born at a low birth weight.[43] Due to several life-threatening medical problems caused by T18, many fetuses die before birth or within the first month after delivery.[43] Only around 5% to 10% of children live past their first year and they experience severe intellectual disabilities.[43] T18 occurs in about 1 in 5,000 newborns. The rate is higher earlier in gestation, but many fetuses do not survive to term.[43] The chance of having a child with this condition increases with maternal age.[43]
- Patau syndrome (T13) is a chromosomal condition associated with severe intellectual disabilities and physical abnormalities in many parts of the body.[44] Affected individuals often have heart defects, brain or spinal cord abnormalities, very small or poorly developed eyes (microphthalmia), extra fingers or toes, an opening in the lip (i.e., cleft lip) with or without an opening in the roof of the mouth (i.e., cleft palate), and hypotonia.[44] Due to several life-threatening medical problems caused by T13, many infants die within their first few days or weeks of life.[44] Only 5% to 10% of children live past their first year.[44] T13 occurs in about 1 in 16,000 newborns.[44] The chance of having a child with T13 increases with maternal age.[44]
- Common sex chromosome aneuploidies include Klinefelter syndrome (47,XXY) and Turner syndrome (45,X).
  - 47,XXY is a chromosomal condition in males that can affect physical and intellectual development.[45] Most commonly, affected individuals are taller than average and are infertile. However, the signs and symptoms of 47,XXY vary among boys and men.[45] In some cases, the condition is mild and is not diagnosed until puberty or adulthood. Researchers estimate that up to 75% of affected individuals are never diagnosed.[45] 47,XXY affects about 1 in 650 newborn boys.[45]
  - 45,X is a chromosomal condition that affects development in girls and women.[46] The most common feature of 45,X is short stature, which becomes evident by about age 5.[46] An early loss of ovarian function (i.e., ovarian hypofunction or premature ovarian failure) is also very common.[46] The ovaries develop normally at first, but egg cells (oocytes) usually die prematurely and most ovarian tissue degenerates before birth.[46] Many affected girls do not undergo puberty unless they receive hormone therapy and most are infertile.[46] This condition occurs in about 1 in 2,500 newborn girls, but is much more prevalent among fetuses that do not survive to term.[46]

Technology of Interest

Cell-free DNA (cfDNA) screening is a type of noninvasive prenatal testing or screening (NIPT) used to determine the risk that a fetus has certain genetic abnormalities.[2] cfDNA testing analyzes fragments of fetal DNA present in maternal blood[2] and is considered noninvasive compared with traditional testing methods such as amniocentesis or chorionic villus sampling. The cfDNA in a maternal blood sample can be screened for T21, T18, and T13, aneuploidies of the sex chromosomes (e.g., 47,XXY and 45,X[1]), and other chromosomal abnormalities (e.g., microdeletions). Microdeletions are chromosomal changes in which a small amount of genetic material on a single chromosome has been deleted. Microdeletions are rare, with a prevalence of 1 in 2,000 to 1 in 100,000 in the U.S., and include DiGeorge syndrome (22q11.2 deletion syndrome), 1p36 deletion syndrome, Prader-Willi syndrome (15q11.2 microdeletion), Angelman syndrome (15q11.2 microdeletion), Cri-du-chat syndrome (5p- syndrome), Wolf-Hirschhorn

syndrome (4p- syndrome), Jacobsen syndrome (11q24.1 deletion syndrome), and Langer-Giedion syndrome (8q24.11 deletion syndrome).[47-49]

The effectiveness of cfDNA screening has mainly been evaluated in women already known to have a higher risk of pregnancies with a chromosomal abnormality.[1,7] The effectiveness of such tests in women at low or unknown risk is more limited.[7] The American College of Obstetricians and Gynecologists (ACOG) has stated that the positive predictive value of cfDNA screening is better for individuals with an increased risk of having a child with a chromosomal disorder.[1] ACOG recommends that "all women be offered the option of aneuploidy screening or diagnostic testing for fetal genetic disorders, regardless of maternal age."[1] ACOG does not recommend any particular test or screening strategy because all available tests have advantages and disadvantages that may make them more or less appropriate for a particular woman, given her needs and preferences.[1] Therefore, ACOG recommends that obstetric care providers be prepared to discuss the benefits, risks, and limitations of all types of screening tests,[1] including cfDNA screening tests available in the U.S. (Table 1), with their patients.

Table 1. Cell-free DNA Screening Tests Available in the U.S.

Cell-free DNA Screening Test Name	Manufacturer
ClariTest (aneuploidy screening)	GenPath Diagnostics
Harmony Prenatal Test	Roche
informaSeq	Integrated Genetics
MaterniT21 PLUS (Core and ESS)	Integrated Genetics
Panorama	Natera
Prequel Prenatal Screen	Myriad Genetics
QNatal Advanced	Quest Diagnostics
Veracity	NIPD Genetics
verifi and verifi Plus Prenatal Test	Illumina
VisibiliT	Sequenom

In the U.S., cfDNA screening for individuals with a high risk of fetal aneuploidy is covered by most commercial and public insurance plans.[50] Some insurance companies, including Anthem Blue Cross Blue Shield and Cigna, now cover cfDNA screening for all pregnancies.[50] However, clinical practice guidelines vary in their recommendations, citing challenges with cost and the positioning of cfDNA in the screening and diagnostic pathways.[26,51] Therefore, questions remain as to whether cfDNA tests should be used universally in the general obstetric population or only in cases of increased risk of aneuploidy (e.g., increased maternal age or family history of a particular genetic disorder).

Policy Context

cfDNA testing is used for prenatal screening for common chromosomal abnormalities. Uncertainty exists regarding the appropriateness of cfDNA screening for some populations, including those at low risk for common fetal genetic abnormalities. This topic was selected for a health technology assessment due to

medium concerns for the safety and efficacy of cfDNA screening in the general population and high concerns for cost.

This evidence review will help inform the State of Washington's independent Health Technology Clinical Committee as it determines coverage regarding cfDNA screening for pregnant women not known to be at high risk for pregnancies with chromosomal abnormalities.

Washington State Utilization and Cost Data

Populations

The cfDNA analysis combined utilization and cost data from the following Washington agencies: the Public Employees Benefit Board Uniform Medical Plan (PEBB/UMP), Medicaid Managed Care (MCO), and Fee-for-Service (FFS). The Department of Labor and Industries Workers' Compensation Plan reported no cfDNA utilization.

Population inclusion criteria specified females incurring at least one target Current Procedural Terminology code from Table I. Utilization counts excluded denied claims. Medicaid analysis excluded individuals with dual eligibility. PEBB/UMP analysis excluded all Medicare claims. The analysis period contained 4 calendar years (CY) of claims data, 2015 through 2018; each year included a minimum of 90 days of claims runout. All chart and graph analyses are by calendar year.

Table I. Targeted CPT Codes: cfDNA Screens

CPT	Procedure Code Description
0009M	Fetal aneuploidy (T21 and T18) DNA sequence analysis of selected regions using maternal plasma; algorithm reported as a risk score for each trisomy; start Jan 1, 2016
81420	Fetal chromosomal aneuploidy (e.g., T21, monosomy X) genomic sequence analysis panel; circulating cell-free fetal DNA in maternal blood; must include analysis of chromosomes 13, 18, and 21; start Jan 1, 2015
81507	Fetal aneuploidy (T21, T18, and T13) DNA sequence analysis of selected regions using maternal plasma; algorithm reported as a risk score for each trisomy; start Jan 1, 2014

Abbreviations. cfDNA: cell-free DNA; CPT: Current Procedural Terminology.

Methods

Participating Washington agencies use predetermined claims extract formats that include more than 45 individual claims-related fields. The claims extracts included all instances of target CPTs for cfDNA. Each agency analysis included examination by date, age cohort (<35 years old and ≥ 35 years old), CPT code (0009M, 81420, and 84507), payment status (paid or denied), contracting (Managed Care or FFS), billing pattern (professional and outpatient), and select diagnosis (ICD-9 and ICD-10).

Findings

Table II. CY 2015 to 2018: Medicaid Managed Care Organizations and Medicaid Fee-for-Service Utilization, cfDNA Screens (CPT 81507, 81420, 0009M)

	2015	2016	2017	2018
Overall unique individuals by year	1,550	3,230	4,454	3,129
Unique individuals by age cohort: <35 years old	805	1,953	2,781	1,728
≥35 years old	745	1,277	1,673	1,401
Unique individuals by Medicaid program: MCO	1,428	3,019	3,893	2,493
FFS	123	211	561	636
Count of total cfDNA screens	1,557	3,257	4,483	3,154
Average paid/cfDNA screen	$951	$852	$598	$482
Total paid for all cfDNA screens	$1,480,626	$2,775,372	$2,679,206	$1,520,648
Total Washington State Medicaid births[1]	42,666	43,398	41,463	41,940[2]

Abbreviations. cfDNA: cell-free DNA; CPT: Current Procedural Terminology; CY: calendar year; FFS: fee-for-service; MCO: managed care organization. Notes. [1] Total Medicaid births from "Characteristics of Washington State Medicaid Women Who Gave Birth," prepared for the Health Care Authority by DSHS Research and Data Analysis, May 9, 2019, Womenhealth@hcs.wa.gov. Medicaid births account for almost half of all Washington State births. [2] Estimated total Washington State Medicaid births for 2018. Estimation based on an anticipated 1.5% increase from 2017 births. "Characteristics of Washington State Medicaid Women Who Gave Birth," prepared for the Health Care Authority by DSHS Research and Data Analysis, May 9, 2019.

Table III. CY 2015 to 2018: Medicaid MCO and FFS Rate of Annual cfDNA Screens Per 1,000 Medicaid Births

2015	2016	2017	2018
36	75	108	75

Abbreviations. cfDNA: cell-free DNA; CY: calendar year; FFS: fee-for-service; MCO: managed care organization.

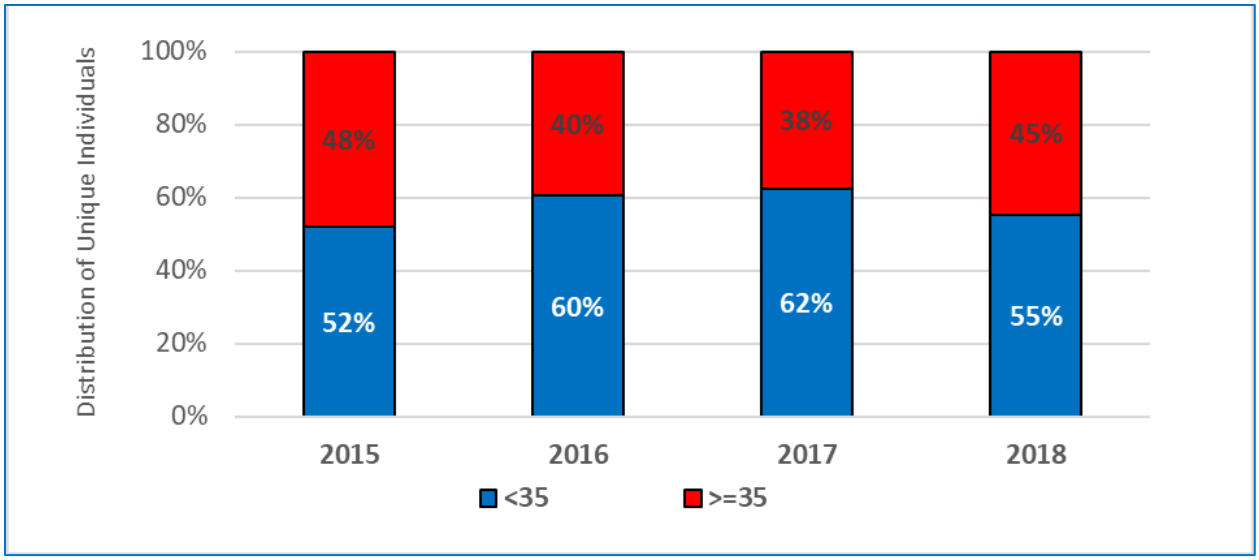


Figure I. CY 2015 to 2018: Medicaid MCO and FFS Distribution of Unique Individuals with cfDNA Screens by Age Cohort, All Diagnoses

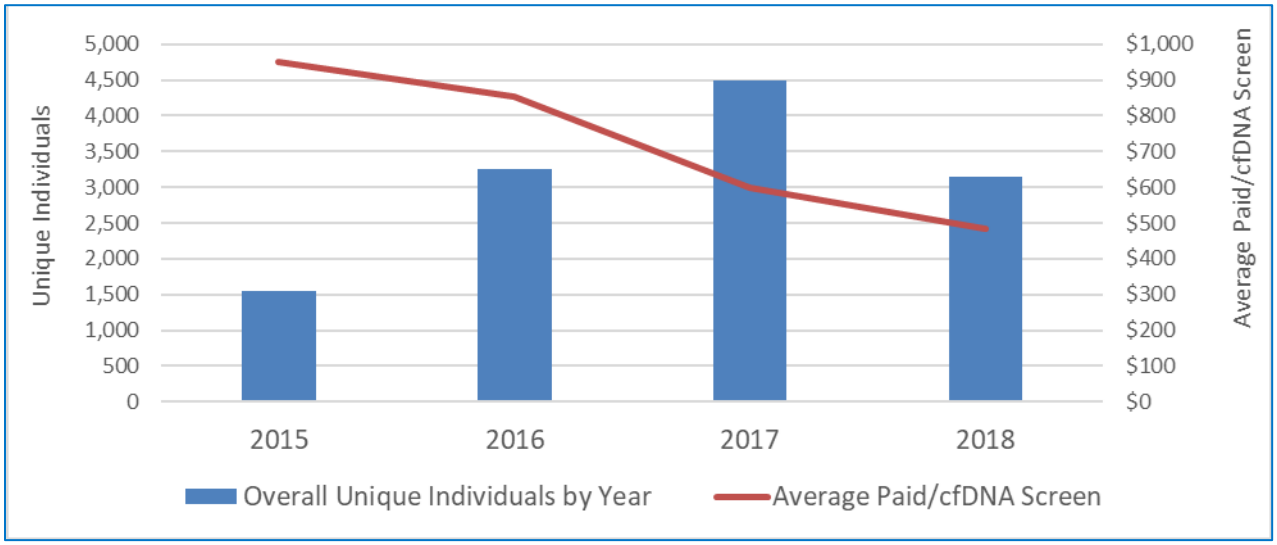


Figure II. CY 2015 to 2018: Medicaid MCO and FFS Unique Individuals with a cfDNA Screen and Average Paid per Screen

Table IV. CY 2015 to 2018: Medicaid MCO and FFS Count of Unique Individuals with cfDNA Screens and a Diagnosis of Supervision of High-risk Pregnancy O09.xx or V23 to V23.99 (see Table V) by Age Cohort Distribution as a Percentage of all Individuals with cfDNA Screens

Unique Individual	2015	2016	2017	2018
<35 years old	44 (5%)	184 (9%)	278 (10%)	246 (14%)
≥35 years old	229 (31%)	1,056 (83%)	1,343 (80%)	1,109 (79%)

Abbreviations. cfDNA: cell-free DNA; CY: calendar year; FFS: fee-for-service; MCO: managed care organization.

Table V. ICD-9 and ICD-10 Diagnoses: Supervision of High-risk Pregnancy

ICD-10	Diagnosis Code Description
O09–O09.0xx	Supervision of high-risk pregnancy
O09.1–O09.13	Supervision of pregnancy with history of ectopic pregnancy
O09.2–O09.299	Supervision of pregnancy with other poor reproductive or obstetric history
O09.3–O09.33	Supervision of pregnancy with other poor reproductive or obstetric history
O09.4–O09.43	Supervision of pregnancy with grand multiparity, unspecified trimester
O09.5–O09.529	Supervision of elderly primigravida and multigravida
O09.6–O09.629	Supervision of young primigravida and multigravida
O09.7–O09.73	Supervision of high-risk pregnancy due to social problems
O09.8–O09.899	Supervision of other high-risk pregnancies
O09.9–O09.93	Supervision of high-risk pregnancy, unspecified
O09.A–O09.A3	Supervision of pregnancy with history of molar pregnancy
ICD-9	**Diagnosis Code Description**
V23–V23.0	Supervision of high-risk pregnancy
V23.1	Supervision of high-risk pregnancy with history of trophoblastic disease
V23.2	Supervision of high-risk pregnancy with history of abortion
V23.3	Supervision of high-risk pregnancy with history of grand multiparity
V23.4–V23.49	Supervision of high-risk pregnancy with other poor obstetric history
V23.5	Supervision of high-risk pregnancy with other poor reproductive history
V23.7	Supervision of high-risk pregnancy with insufficient prenatal care
V23.8–V23.8.9	Supervision of other high-risk pregnancy
V23.9	Supervision of unspecified high-risk pregnancy

Abbreviation. ICD: International Statistical Classification of Diseases and Related Health Problems.

Table VI. CY 2015 to 2018: PEBB/UMP (No Medicare); Utilization: cfDNA Screens (CPT 81507, 81420, 0009M)

	2015	2016	2017	2018
Overall unique individuals by year	167	363	705	696
Unique individuals by age cohort: <35 years old	41	125	310	293
≥35 years old	126	238	395	404
Distribution of unique individuals by age cohort:				
<35 years old	25%	34%	44%	42%
>=35 years old	75%	66%	56%	58%
Count of total cfDNA screens	167	363	708	700
Average amount paid/cfDNA screen	$787	$733	$616	$553
Total paid for all cfDNA screens	$111,730	$235,307	$385,017	$376,793
Total annual PEBB/UMP births	1,874	2,036	2,041	2,048

Abbreviations. cfDNA: cell-free DNA; CPT: Current Procedural Terminology; CY: calendar year; PEBB/UMP: Public Employees Benefit Board Uniform Medical Plan.

Table VII. PEBB/UMP Rate of Annual cfDNA Screens Per 1,000 PEBB-UMP Births

2015	2016	2017	2018
89	178	347	342

Abbreviations. cfDNA: cell-free DNA; PEBB/UMP: Public Employees Benefit Board Uniform Medical Plan.

Table VIII. CY 2015 to 2018: PEBB/UMP (No Medicare) Count of Unique Individuals with cfDNA Screens and a Diagnosis of Supervision of High-risk Pregnancy O09.xx or V23 to V23.99 (see Table V) by Age Cohort Distribution of all Individuals with a cfDNA Screen

Unique Individual	2015	2016	2017	2018
<35 years old	16 (16%)	39 (26%)	45 (14%)	39 (13%)
≥35 years old	78 (41%)	222 (83%)	300 (76%)	313 (77%)

Abbreviations. cfDNA: cell-free DNA; CY: calendar year; PEBB/UMP: Public Employees Benefit Board Uniform Medical Plan.

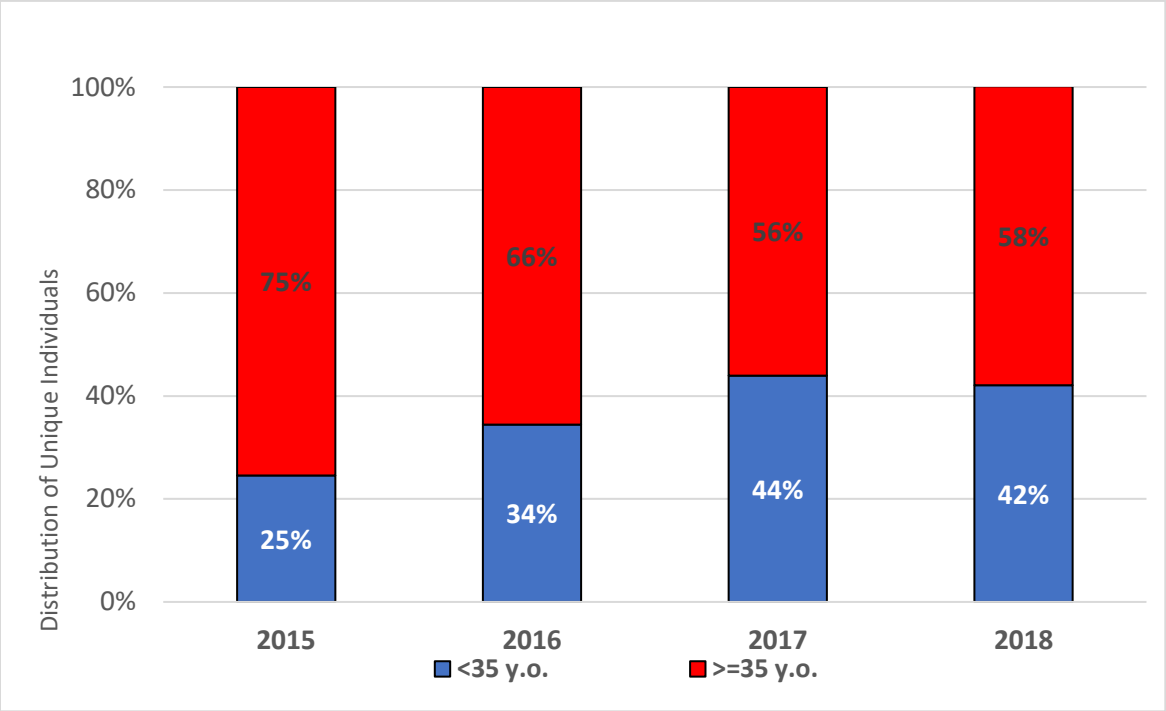


Figure III. CY 2015 to 2018: PEBB/UMP (No Medicare) Distribution of Unique Individuals with cfDNA Screens by Age Cohort, All Diagnoses

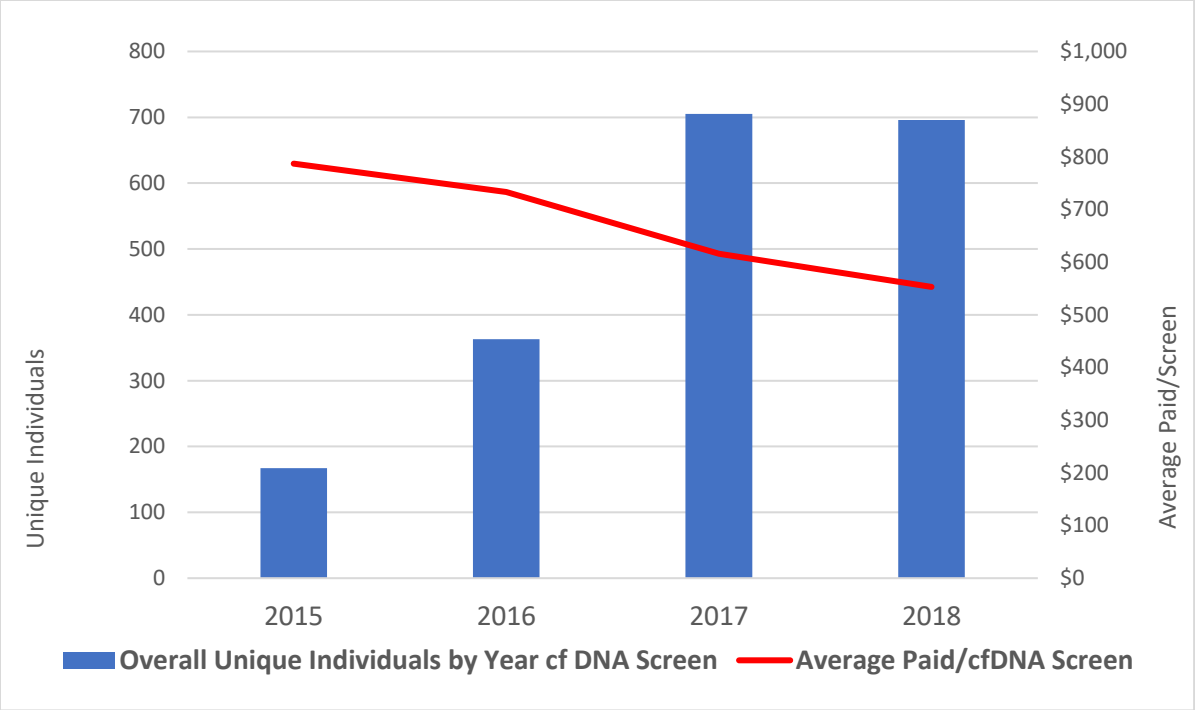


Figure IV. CY 2015 to 2018: PEBB/UMP (No Medicare) Unique Individuals with a cfDNA Screen and Average Paid per Screen

Methods

This evidence review is based on the final key questions (KQs) published on August 6 and updated on August 26, 2019.[3] The draft KQs were available for public comment from July 10 to July 23, 2019, and appropriate revisions were made to the KQs based on the comments and responses.[4] All public comments received and a table of responses can be found on the Washington Health Technology Assessment website. The draft report will be open for public comment between October 23 and November 21, 2019, and appropriate revisions based on comments will be made and posted to the program's website. The draft report will be peer reviewed by experts in genetics, perinatology, and genetic counseling and appropriate revisions will be reflected in the final report. The PICO statement (population, intervention, comparator, outcome), along with the setting, study design, and publication factors) that guided development of the KQs and study selection are presented in table 2 below.

Key Questions

1. What is the evidence on the efficacy and effectiveness of using cfDNA in pregnant individuals not known to be at high risk for chromosomal abnormalities:

    a. For trisomies 21, 18, and 13, compared to active screening approaches, including standard screening with serum biomarkers and ultrasound, screening with another cfDNA screening test, question-based screening, or invasive diagnostic testing?

    b. For common sex chromosome aneuploidies, any active screening approach, screening with another cfDNA screening test, no screening, or invasive diagnostic testing?

2. What direct harms are associated with screening using cfDNA in pregnant individuals not known to be at high risk for chromosomal abnormalities:

    a. For trisomies 21, 18, and 13, compared to active screening approaches, including standard screening with serum biomarkers and ultrasound, screening with another cfDNA screening test, question-based screening, or invasive diagnostic testing?

    b. For common sex chromosome aneuploidies, any active screening approach, screening with another cfDNA screening test, no screening, or invasive diagnostic testing?

3. Do important efficacy/effectiveness outcomes or direct harms of screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA vary for the mother and fetus or infant by:
    a. Maternal characteristics (e.g., age)
    b. Singleton or multifetal pregnancy
    c. Timing of screening (e.g., gestational age)

4. What are the cost-effectiveness and other economic outcomes of screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA in pregnant individuals not known to be at high risk for chromosomal abnormalities?

Contextual questions are not shown in the analytic framework. To address contextual questions, we relied on recent high-quality systematic reviews.

Contextual Question 1. What are the benefits and harms of screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA in pregnant individuals known to be at high risk for chromosomal abnormalities?

Analytic Framework

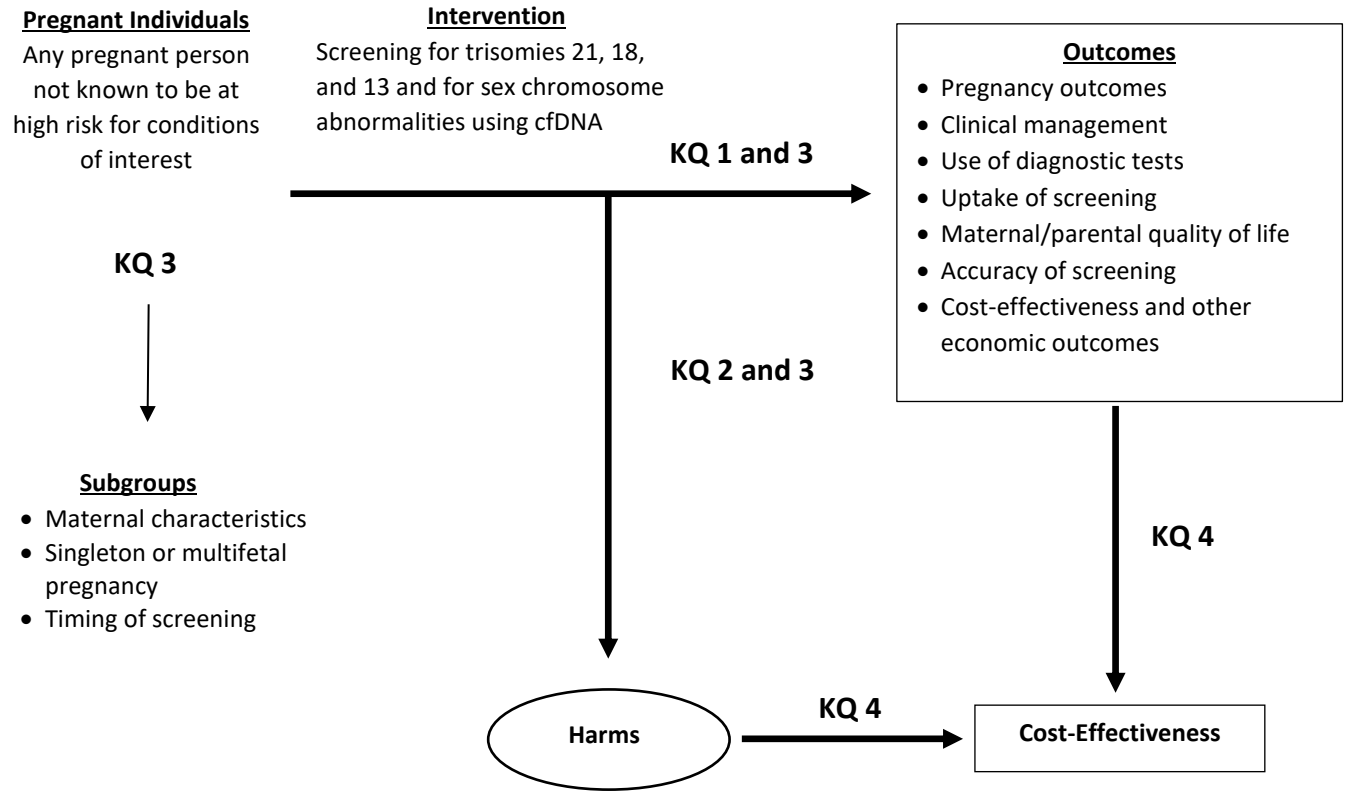


Figure 1. Analytic Framework

Eligible Studies

Table 2 summarizes the study inclusion and exclusion criteria.

Table 2. Key Study Inclusion and Exclusion Criteria

Study Component	Inclusion	Exclusion
Populations	• Pregnant individuals of any age, ethnicity, and gestational age with a singleton or multifetal (monochorionic and dichorionic) pregnancy, who are not known as being at high risk for the target fetal conditions (i.e., an unselected population that is representative of the general population) • We also included studies of mixed-risk populations, where outcomes were reported by the level of risk	• Studies including only pregnant individuals known to be at high-risk (e.g., past history or identified as high risk through prenatal screening) • Studies in which the population risk is undetermined • Studies including only patients undergoing preimplantation testing of embryos for IVF
Interventions	• Screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA	• Screening with cfDNA or other NIPT technologies for other chromosomal abnormalities or genetic conditions • Studies with an outdated cfDNA screening test or a cfDNA screening test that is not available in the U.S.
Comparators	• For trisomies, active screening approaches, including standard screening with serum biomarkers and ultrasound, screening with another cfDNA screening test, or question-based screening • For common sex chromosome aneuploidies, any active screening approach, screening with another cfDNA screening test, or no screening • Invasive diagnostic testing (e.g., amniocentesis)	• Studies without a comparator intervention • Studies with indirect comparisons • Studies with an outdated comparator or a comparator intervention that is not available in the U.S.
Outcomes	• Primary outcomes: pregnancy outcomes; use of cfDNA results for clinical management (e.g., further diagnostic testing, counseling) • Secondary outcomes: uptake of cfDNA screening; maternal/parental/family quality of life, including satisfaction (measured with validated instruments) • Safety: harms directly related to screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA tests (e.g., misclassification, psychosocial harms) • Indirect outcomes: measures of cfDNA screening test performance • Economic: cost-effectiveness outcomes (e.g., cost per improved outcome) or cost-utility outcomes (e.g., cost per QALY, ICER)	• Other outcomes • Cost of testing from studies performed in non-U.S. countries • Cost of testing from studies performed in the U.S. older than 5 years

Study Component	Inclusion	Exclusion
Setting	• Any outpatient or inpatient clinical setting in countries categorized as very high on the UN Human Development Index52	• Nonclinical settings (e.g., studies conducted using libraries of plasma samples) • Countries categorized other than very high on the UN Human Development Index52

Study design	• Key Questions 1–4
	  ○ Randomized controlled trials
	  ○ Systematic reviews of randomized controlled trials
	  ○ Nonrandomized, comparative studies
	• Additional studies/data for Key Questions 2 and 3 (harms)
	  ○ Nonrandomized studies without a comparator and with 10 or more participants
	• Additional studies/data for Key Question 4
	  ○ Cost-effectiveness studies and other formal comparative economic evaluations
	  ○ Systematic reviews of cost-effectiveness studies and other formal comparative economic evaluations
		• Abstracts, conference proceedings, posters, editorials, letters
		• Case reports and case series with fewer than 10 subjects (for harms only)
		• Proof-of-principle studies (e.g., algorithm modification)
		• Studies with harms outcomes for a test that is not included in Key Question 1
		• Systematic reviews that are superseded by a more comprehensive or high-quality systematic review

Publication	• Studies in peer-reviewed journals, technology assessments, or publicly available FDA or other government reports • Published in English • Published from 2007 through July 2019	• Studies whose abstracts do not allow study characteristics to be determined • Studies that cannot be located • Duplicate publications of the same study that do not report different outcomes or follow-up times, or single site reports from published multicenter studies • Studies in languages other than English • Studies published prior to 2007

Abbreviations. cfDNA: cell-free DNA; FDA: U.S. Food and Drug Administration; ICER: incremental cost-effectiveness ratio; QALY: quality-adjusted life year; UN: United Nations.

Data Sources and Searches

We conducted searches of the peer-reviewed published literature using multiple electronic databases. The time periods for searches were:

- Ovid MEDLINE, Ovid MEDLINE In-Process & Other Non-Indexed Citations, and Ovid MEDLINE Epub Ahead of Print: from 2007 to July 8, 2019
- Cochrane Library databases (Cochrane Database of Systematic Reviews and Cochrane Central Register of Controlled Trials): from 2007 to July 8, 2019
- Scopus: from 2007 to July 9, 2019

- NHS Economic Evaluation Database (EED) and the National Institute for Health Research (NIHR) Health Technology Assessment Program (HTA) database: from 2007 to July 9, 2019

Randomized controlled trials (RCTs) and systematic reviews (with and without meta-analyses) and health technology assessments that included RCTs were considered for KQs 1 to 4. Nonrandomized comparative studies and nonrandomized studies without a comparator were considered for KQs 1 and 3 and for the harm-related aspects of KQs 2 and 3 if evidence for the intervention was included in KQ 1. For KQ4, we also considered cost-effectiveness studies and other comparative economic evaluations, as well as systematic reviews (with and without meta-analyses) reporting economic outcomes.

The Ovid MEDLINE search strategy is shown in Appendix A. We also screened reference lists of relevant studies and used lateral search functions, such as *related articles* and *cited by*. We searched the following additional sources:

- Agency for Healthcare Research and Quality (AHRQ)
- National Institute for Health and Care Excellence (NICE) – Evidence
- Veterans Administration Evidence-based Synthesis Program

We searched these sources for systematic reviews and clinical practice guidelines using the same search terms outlined for the evidence search. In addition, we conducted a search of GuidelineCentral (www.guidelinecentral.com) and the Guidelines International Network guidelines library (https://g-i-n.net/home) in August 2019, as well as the websites of professional organizations for relevant guidelines. In these searches, we used terms related to prenatal screening and considered guidelines published in the past 5 years (January 2014 to 2019) for inclusion.

Using Google Search, we conducted a general internet search for appropriate published studies and relevant gray literature. We also searched the Medicare Coverage Database for National Coverage Determinations and Local Coverage Determinations located on the Centers for Medicare & Medicaid Services website for literature relevant to the State of Washington. And we searched the Aetna, Cigna, and Regence websites for private payer coverage policies.

To identify relevant ongoing clinical trials, we searched the online database of clinical trials (ClinicalTrials.gov) maintained by the National Library of Medicine at the National Institutes of Health for terms related to prenatal screening and cfDNA. The information in this database was provided by the sponsor or principal investigator of each study. Studies are generally registered in the database when they begin and information is updated as the study progresses. We also considered studies submitted during the public comment process for possible inclusion.

Screening

We (VK and BS) independently screened titles and abstracts and reached agreement on exclusion through discussions. For studies on which we could not agree, we performed full-text reviews for inclusion criteria (Appendix H lists the excluded studies, with reasons). We then discussed the inclusion criteria until we reached agreement (Figure 2). Any remaining disagreements were settled by a third independent researcher (CH).

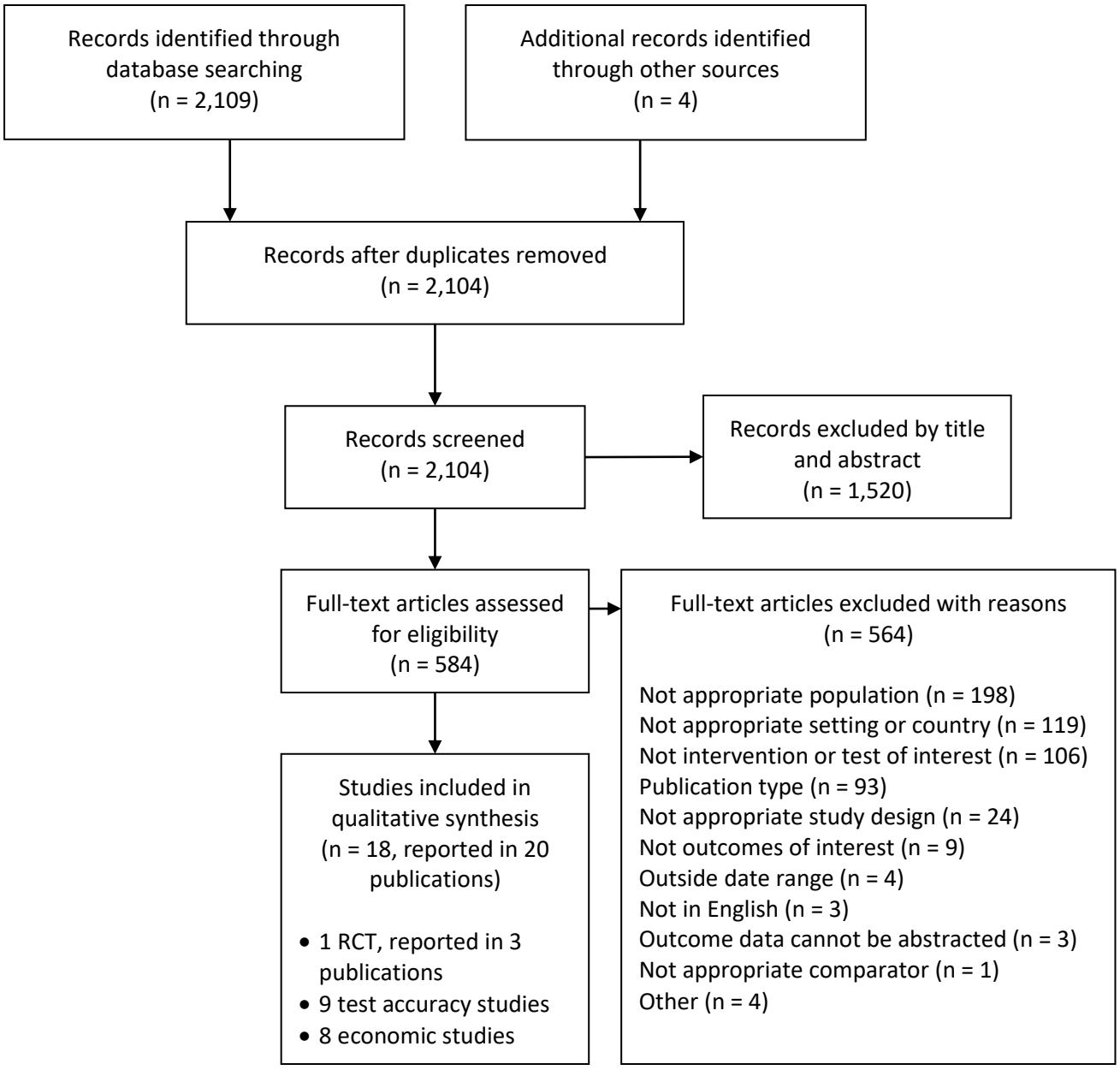


Figure 2. PRISMA Study Flow Diagram

Data Abstraction and Quality Assessment

We used standardized procedures to extract relevant data from each of the included trials and fully cross-checked all entered data for accuracy.

We (VK and BS) evaluated each eligible study for methodological risk of bias (Appendix D) and held discussions to reach agreement on these assessments. Any remaining disagreement was settled by a third independent researcher (CH). Each trial was assessed using Center instruments adapted from national and international standards and assessments for risk of bias.[53-58] A rating of high, moderate, or low risk of bias was assigned to each study based on adherence to recommended methods and the potential for internal and external biases. The risk-of-bias criteria for the study types are shown in Appendix B.

We (ME and BS) evaluated the methodological quality of eligible clinical practice guidelines. Any remaining disagreement among these assessments was settled by a third independent researcher (CH). The methodological quality of clinical practice guidelines was rated as good, fair, or poor. The assessment criteria for the methodological quality of the clinical practice guidelines are shown in Appendix B.

Data Analysis and Synthesis

When study authors did not report measures of test performance or the studies included reporting discrepancies, we used MedCalc's diagnostic test evaluation calculator[5] to calculate relevant test performance statistics with 95% confidence intervals (CIs). We originally planned to conduct a meta-analysis of key outcomes of the impact of screening with cfDNA if a sufficient number of studies reported equivalent outcomes at similar timeframes. However, a meta-analysis was not possible because we found only 1 study reporting on the impact of cfDNA screening. We did not conduct a meta-analysis of the test performance of the included tests, but we used RevMan to produce graphical summaries of the test performance measures.[6]

We assigned selected outcomes a summary judgment for the overall quality of evidence (Appendix E) using the system developed by the GRADE Working Group.[59,60] The outcomes were selected from measures of impact and test performance. Specific measures from general domains of interest were selected in a post-hoc manner based on the outcomes available from the included studies.

The GRADE system[60] defines the overall quality of a body of evidence for an outcome in the following manner:

- **High**: Raters are very confident that the estimate of the effect of the intervention on the outcome lies close to the true effect. Typical sets of studies are RCTs with few or no limitations, and the effect estimate is likely stable.
- **Moderate**: Raters are moderately confident in the estimate of the effect of the intervention on the outcome. The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is different. Typical sets of studies include RCTs with some limitations or well-performed nonrandomized studies with additional strengths that guard against potential bias and have large estimates of effects.
- **Low**: Raters have little confidence in the estimate of the effect of the intervention on the outcome. The true effect may be substantially different from the estimate of the effect. Typical

sets of studies include RCTs with serious limitations or nonrandomized studies without special strengths.

- **Very low**: Raters have no confidence in the estimate of the effect of the intervention on the outcome. The true effect is likely to be substantially different from the estimate of the effect. Typical sets of studies include nonrandomized studies with serious limitations or inconsistent results across studies.
- **Not applicable**: Researchers did not identify any eligible articles.

We used GRADEpro software to develop the GRADE tables for test performance.[61]

Evidence Summary

Our searches returned a total of 2,109 records, and we added an additional 4 records from other sources. The 4 additional records arose from reviewing reference lists and relevant websites. We also checked the reference lists of relevant systematic reviews.[7,62-90]

We found no additional studies, beyond those identified in electronic databases, through Google and gray literature searches. After duplicate studies were removed, 2,104 records remained. Of these, 584 required full-text review to determine eligibility. Of these, 1 RCT (in 3 publications) and 9 test accuracy studies met the inclusion criteria for KQs 1, 2, and 3.[8-17] In addition, 8 economics studies met the inclusion criteria for KQ 4.[18-25]

Measures of Test Performance

Health screening is the process of identifying people who may have an increased chance of a disease or condition. The screening procedure itself does not definitively diagnose an illness. People who have a positive result from a screening test should be offered further evaluation, including subsequent diagnostic tests or procedures. To understand how well a screening test identifies people at increased risk, a number of measures can be used (details are provided in Appendix G):

- Sensitivity (or detection rate): probability that a test result will be positive when the disease is present (true positive rate)[5]
- Specificity (or true-negative rate): probability that a test result will be negative when the disease is not present (true negative rate)[5]
- Positive predictive value (PPV): probability that the disease is present when the test is positive[5]
- Negative predictive value (NPV): probability that the disease is not present when the test is negative[5]

Generally, a good screening test has high degrees of sensitivity and specificity. The predictive value is determined by the sensitivity and specificity of the test and the prevalence of the condition in the population being tested.[91] The more sensitive a test, the less likely it is that an individual with a negative test actually has the condition and thus the greater the negative predictive value.[91] The more specific the test, the less likely it is that an individual with a positive test does not have the condition and the greater the positive predictive value.[91]

While sensitivity and specificity are generally characteristics of the test itself, PPV is a function of both the test characteristics and the underlying risk of the condition (prevalence) in the particular population being tested. For rare conditions, a large proportion of those with positive screening tests will inevitably

be found not to have the condition upon further diagnostic testing.[91] Simply, the less prevalent a condition, the more likely a positive test is a false positive (FP). For example, if the prevalence of a condition is 0.1%, a test with a sensitivity of 100% and a specificity of 99.5% will have a PPV of 16.7%. The same test will have a PPV of 66.7% if the prevalence of the condition is 1.0%.

To increase the PPV of a screening test, programs often target the screening test to those at high risk of developing the condition, based on considerations such as demographic factors or medical history.[91]

Other measures of interest include the rates of FPs and false negatives (FNs). For prenatal screening, the implications of a FP test include unnecessary invasive testing and for a FN test, implications include birth of an infant with increased care needs and the loss of choice as to whether to terminate the pregnancy.

Contextual Question 1

In order to contextualize the performance of cfDNA screening in the general obstetric population, we searched for a recent systematic review of the benefits and harms of screening for trisomies 13, 18, and 21 and for common sex chromosome aneuploidies using cfDNA in pregnant individuals known to be at high risk for chromosomal abnormalities. In 2017, a Cochrane review evaluated the diagnostic accuracy cfDNA screening as a first-tier test in unselected populations of pregnant women undergoing aneuploidy screening or as a second-tier test in pregnant women considered to be high risk after first-tier screening for common fetal aneuploidies.[7] Eligible studies included pregnant women of any age, ethnicity, and gestational age with a singleton or multifetal pregnancy.[7] Study participants had to have had a screening test for fetal aneuploidy by cfDNA and by a reference standard such as fetal karyotype or medical records from birth.[7] Each of the included studies had some risk of bias, but applicability concerns were generally low.[7]

The Cochrane review included 65 studies of cfDNA screening (using massively parallel shotgun sequencing [MPSS] and targeted massively parallel sequencing [TMPS] technologies) in pregnant women. Of the 65 studies, 42 recruited women at high risk, 5 recruited an unselected population, and 18 recruited cohorts with a mix of prior risk of fetal aneuploidy. Overall, the review found:

- The cfDNA test failure rate (i.e., a "no-call" result) ranged from 0% to 25% in the 46 studies reporting this outcome.[7] When only studies with high-risk women were included, the cfDNA test failure rate ranged from 0.4% to 25%.
- In high-risk populations, MPSS was used to assess the risk of T21 (n = 30 studies), T18 (n = 28 studies), T13 (n = 20 studies), and 45,X (n = 12 studies). Sensitivity and specificity were calculated in a pooled analysis (1,048 T21 cases, 332 T18 cases, 128 T13 cases, and 15,797 unaffected pregnancies; Table 3).
- In high-risk populations, TMPS was used to assess the risk of T21 (n = 6 studies), T18 (n = 5 studies), T13 (n = 2 studies), and 45,X (4 studies). Sensitivity and specificity were calculated in a pooled analysis (246 T21 cases, 112 T18 cases, 20 T13 cases, and 4,282 unaffected pregnancies; Table 3).
- Indirect comparisons of MPSS and TMPS for the ability of the tests to assess the risk of T21, T18, and 45,X showed no statistical differences in clinical sensitivity, clinical specificity, or both. Due to limited data, a comparative meta-analysis of MPSS and TMPS was not possible for T13.[7]

- Few or no studies evaluated cfDNA screening for Triple X syndrome (47,XXX), 47,XXY, and Jacob's syndrome (47,XYY).[7]

Table 3. Pooled Results for cfDNA Screening Tests in High-Risk Populations[7]

Condition	Pooled Sensitivity (95% CI)	Pooled Specificity (95% CI)
MPSS		
T21	99.7% (98.0% to 100%)	99.9% (99.8% to 100%)
T18	97.8% (92.5% to 99.4%)	99.9% (99.8% to 100%)
T13	95.8% (86.1% to 98.9%)	99.8% (99.8% to 99.9%)
45,X	91.7% (78.3% to 97.1%)	99.6% (98.9% to 99.8%)
TMPS		
T21	99.2% (96.8% to 99.8%)	100% (99.8% to 100%)
T18	98.2% (93.1% to 99.6%)	100% (99.8% to 100%)
T13	100% (83.9% to 100%)	100% (98.7% to 100%)
45,X	92.4% (84.1% to 96.5%)	99.8% (98.3% to 100%)

Abbreviations: CI: confidence interval; MPSS: massively parallel shotgun sequencing; T13: trisomy 13; T18: trisomy 18; T21: trisomy 12; TMPS: targeted massively parallel sequencing.

In the Cochrane review, Badeau et al.[7] concluded that "non-invasive prenatal testing methods appear to be sensitive and highly specific for detection of fetal trisomies 21, 18, and 13 in high-risk populations." However, the authors emphasized that invasive fetal karyotyping remains the required diagnostic approach to confirm the presence of a chromosomal abnormality prior to making irreversible decisions relative to the pregnancy outcome.[7]

Key Questions 1 and 2

We found 10 studies, published since 2007, that evaluated the benefits and harms of universal cfDNA screening in pregnant women (

Table 4 and Appendix C, Tables C1, C2, C5-C8).[8-17] Of these, 1 RCT evaluated the impact of cfDNA prenatal screening[8] and 9 studies evaluated the performance of cfDNA as a screening test.[9-17] We rated the risk of bias in these studies as follows:

- One RCT had a moderate risk of bias due to concerns about blinding and allocation concealment.
- Seven test performance studies had a moderate risk of bias due to concerns about patient selection, conflicts of interest, and test interpretation.[10-16]
- Two studies had a high risk of bias due to substantial concerns about limited reporting on the methods used and conflicts of interest.[9,17]

Table 4. Characteristics of Eligible Studies Evaluating cfDNA Screening

Study ID Study Risk of Bias	Study Design Setting	Population	Conditions	Test	Outcomes
Ashoor et al., 2013[9] High	Prospective cohort and case-control U.K. and U.S.	Pregnant women with singleton pregnancies Confirmed cases of T13	T13	Harmony Prenatal (Roche-Ariosa)	Test performance Test failures
Bianchi et al., 2014[10] Moderate	Prospective cohort U.S.	Pregnant women with singleton pregnancies	T21, T18	verifi (Illumina)	Test performance Test failures Pregnancy outcomes
del Mar Gil et al., 2014[11] Moderate	Retrospective cohort U.K.	Pregnant women with twin pregnancies	T21, T18, T13	Harmony Prenatal (Roche-Ariosa)	Test performance Test failures
Kagan et al., 2018[8] Moderate	RCT Germany	Pregnant women with singleton pregnancies	T21, T18, T13	Harmony Prenatal (Roche-Ariosa)	Pregnancy outcomes Risk for trisomies Test failures FP rates Invasive testing
Langlois et al., 2017[12] Moderate	Prospective cohort Canada	Pregnant women with singleton pregnancies	T21, T18, T13	Harmony Prenatal (Roche-Ariosa)	Test performance Test failures Pregnancy outcomes Invasive testing
Nicolaides et al., 2012[13] Moderate	Prospective cohort U.K.	Pregnant women with singleton pregnancies	T21, T18	Harmony Prenatal (Roche-Ariosa)	Test performance Test failures Pregnancy outcomes
Norton et al., 2015[14] Moderate	Prospective cohort U.S., Belgium, Canada, Italy, Netherlands, and Sweden	Pregnant women with singleton pregnancies	T21, T18, T13	Harmony Prenatal (Roche-Ariosa)	Test performance Test failures Pregnancy outcomes
Palomaki et al., 2017[15,92] Moderate	Prospective cohort U.S.	Pregnant women with singleton or twin pregnancies	T21, T18, T13, and 45,X	Panorama (Natera)	Test performance Test failures Pregnancy outcomes

Study ID Study Risk of Bias	Study Design Setting	Population	Conditions	Test	Outcomes
					Invasive testing
Pergament et al., 2014[16] Moderate	Prospective cohort U.S., Czech Republic, Japan, Turkey, Ireland, Spain, and Poland	Pregnant women with singleton pregnancies	T21, T18, T13	Panorama (Natera)	Test performance Test failures
Quezada et al., 2015[17] High	Prospective cohort U.K.	Pregnant women with singleton pregnancies	T21, T18, T13	Harmony Prenatal (Roche-Ariosa)	Test performance Test failures Pregnancy outcomes

Abbreviations. FP: false positive; RCT: randomized controlled trial; T13: trisomy 13; T18: trisomy 18; T21: trisomy 21. Note. Nicolaides et al., 2012[13] and Ashoor et al., 2013[9] reported on the same population, but for different trisomies.

Study Characteristics

The RCT, by Kagan et al.,[8] compared T21 risk assessment by combined FTS with a combination of a detailed ultrasound examination at 11 to 13 weeks' gestation and cfDNA screening, using the Harmony Prenatal test. Women with singleton pregnancies who had a normal first-trimester ultrasound were recruited from a single center in Germany.[8] The fetal aneuploidies of interests were T21, T18, and T13.[8]

Of the 9 test accuracy studies, 7 included women with singleton pregnancies,[9,10,12-14,16,17] 1 included twin pregnancies only,[11] and 1 included singleton and twin pregnancies.[15] Eligible studies were conducted mainly in North America and Europe, with 3 based in the U.K.,[11,13,17] 2 in the U.S.,[10,15] 1 in Canada,[12] 1 in the U.K. with confirmed trisomy cases from the U.S.,[9] and 2 in multiple countries (1 in the U.S., Belgium, Canada, Italy, the Netherlands, and Sweden[14] and 1 in the U.S., Czech Republic, Japan, Turkey, Ireland, Spain, and Poland[16]). Six of the 9 studies evaluated the performance of the Harmony Prenatal (Roche-Ariosa) screening test,[9,11-14,17] 2 evaluated the Panorama (Natera) screening test,[15,16] and 1 evaluated the verifi (Illumina) screening test.[10]

Study Findings

Risk Assessment

In the Kagan et al.[8] RCT, 1,400 pregnant women with a normal first-trimester ultrasound examination were randomized for risk assessment using cfDNA screening and ultrasound findings or conventional FTS, which used maternal and gestational age, fetal nuchal translucency thickness, and maternal levels of serum PAPP-A and free β-hCG.[8] The primary outcome was FP screening rates for T21.[8] Screening for T21 in the intervention group used cfDNA analysis plus ultrasound findings from the examination at 11 to 13 weeks' gestation. In cases of uninformative cfDNA testing, a reserved blood sample was used to compute the risk of T21 using the conventional FTS method. The cfDNA plus ultrasound group had a significantly lower FP screening rate than the conventional FTS group.[8] In the cfDNA group, there were no false-positive cases, whereas the age-adjusted false-positive screening rate in the FTS group was

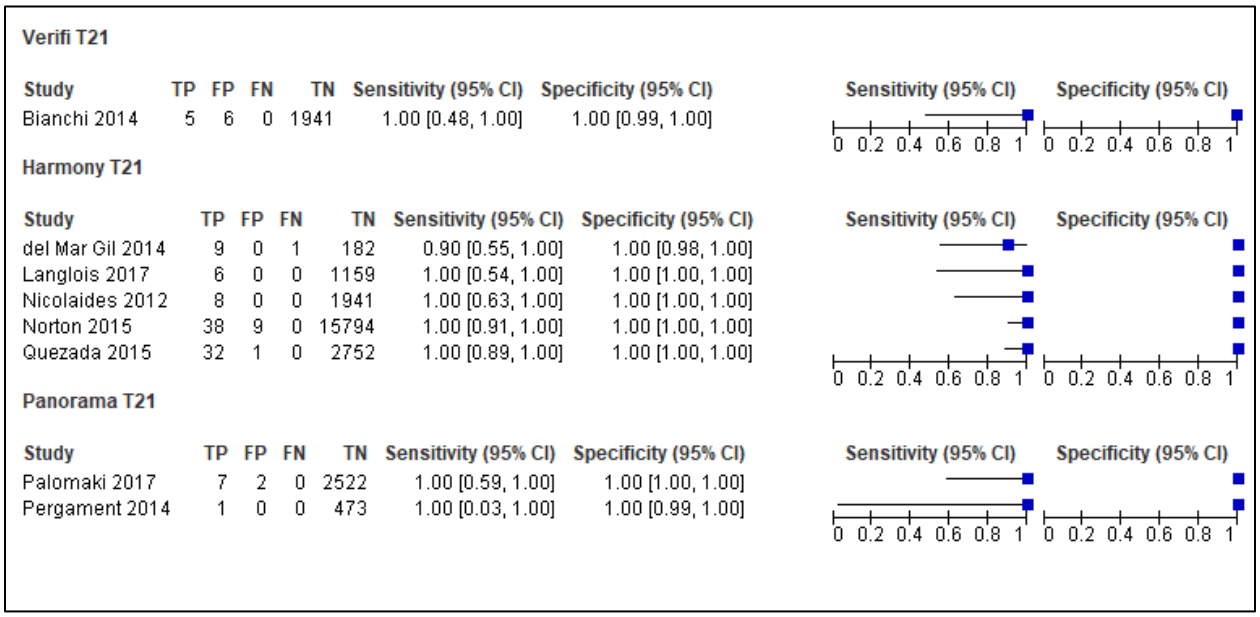
2.5% (*P* value not reported).[8] In the cfDNA plus ultrasound group, the median risk for T21 was 1 in 10,000 and no individual had a risk for T13, T18, or T21 greater than 1:100.[8] In the conventional FTS group, the median risk for T21 was 1 in 3,787 and 17 cases had a risk greater than 1:100.[8] The risk of T21 in the cfDNA plus ultrasound group was significantly lower than in the conventional FTS group (risk above 1:100: 0% cfDNA; 95% CI, 0% to 0.5%; 2.5% FTS; 95% CI, 1.2% to 3.6%; *P* < .001).[8]

Test Performance of Screening Using cfDNA

Test performance was assessed in 9 studies. We extracted the 2x2 data, which allowed us to calculate the sensitivity and specificity and the predictive values of the screening tests (Appendix G presents the measures of test performance).

T21

In general obstetric populations, screening with cfDNA had a median sensitivity of 100% (range, 90.00%-100%) and a median specificity of 99.98% (range, 99.69%-100%) () for T21.



Abbreviations. CI: confidence interval; FN: false negative; FP: false positive; T21: trisomy 21; TN: true negative; TP: true positive.

Figure 3. Sensitivity and Specificity of cfDNA Tests for T21

For T21 screening, the PPVs ranged from 45.45% to 100% (median, 98.48%) and NPVs from 99.45% to 100% (median, 100%) (

Table 5).

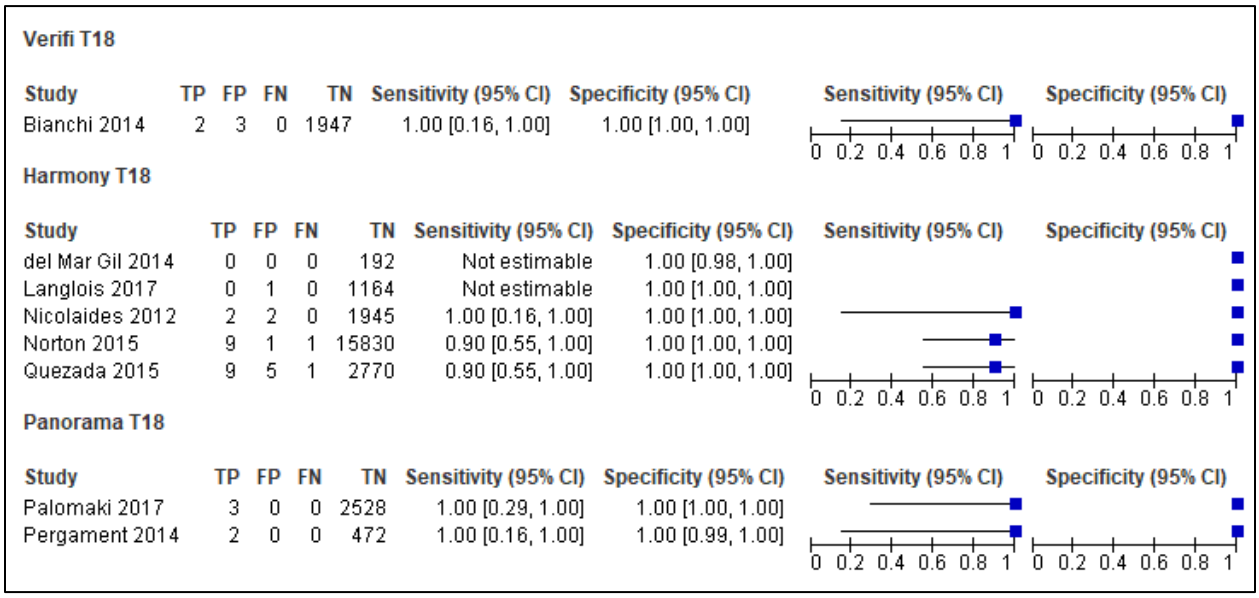
Table 5. Positive and Negative Predictive Values of cfDNA for T21

Study ID	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Bianchi et al., 2014[10]	45.5% (27.3%-64.9%)	100%
del Mar Gil et al., 2014[11]	100%	99.5% (96.6%-99.9%)
Langlois et al., 2017[12]	100%	100%
Nicolaides et al., 2012[13]	100%	100%
Norton et al., 2015[14]	80.9% (68.7%-89.0%)	100%
Palomaki et al., 2017[15,92]	77.8% (46.7%-93.3%)	100%
Pergament et al., 2014[16]	100%	100%
Quezada et al., 2015[17]	97.0% (81.9%-99.6%)	100%

Abbreviation. CI: confidence interval.

T18

In general obstetric populations, screening with cfDNA had a median sensitivity of 100% (range, 90.0%-100%) and a median specificity of 99.95% (range, 99.8%-100%) () for T18.



Abbreviations. CI: confidence interval; FN: false negative; FP: false positive; T18: trisomy 18; TN: true negative; TP: true positive.

Figure 4. Sensitivity and Specificity of cfDNA Tests for T18

For T18, the PPVs ranged from 40.0% to 100% (median, 77.1%) and NPVs from 99.96% to 100% (median, 100%) (

Table 6).

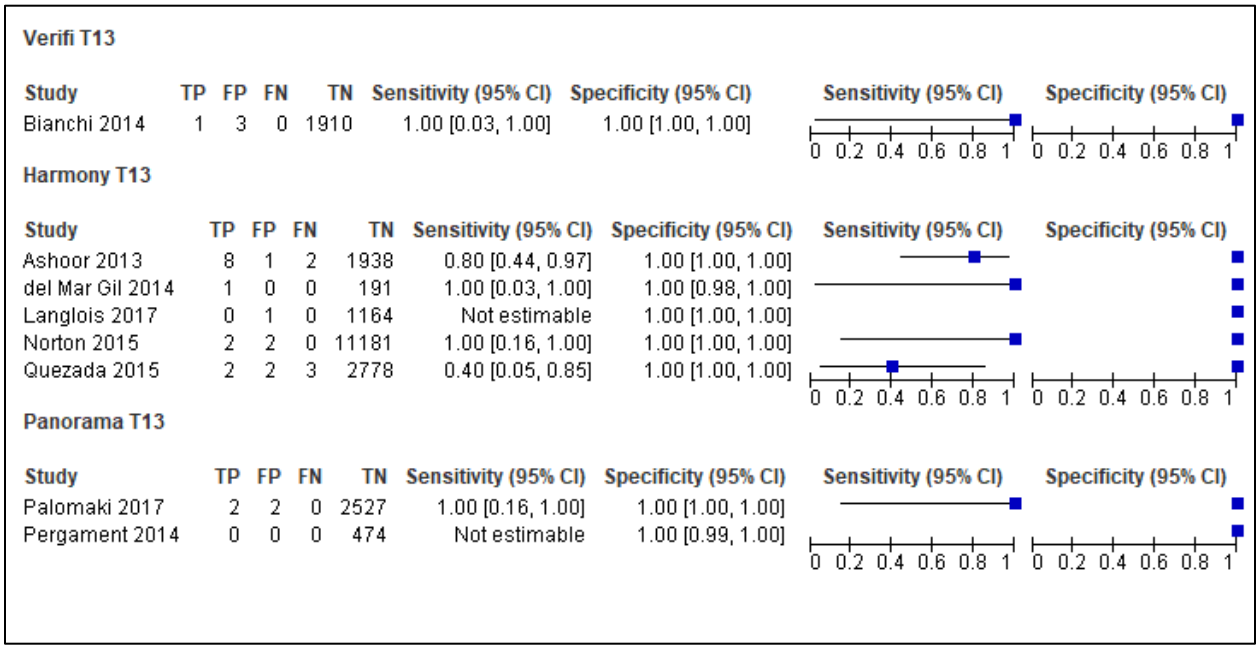
Table 6. Positive and Negative Predictive Values of cfDNA for T18

Study ID	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Bianchi et al., 2014[10]	40.0% (17.7%-67.4%)	100%
del Mar Gil et al., 2014[11]	No cases of T18 occurred	100%
Langlois et al., 2017[12]	No cases of T18 occurred	100%
Nicolaides et al., 2012[13]	50.0% (20.0%-80.0%)	100%
Norton et al., 2015[14]	90.0% (55.6%-98.5%)	99.99 (99.96%-100.0%)
Palomaki et al., 2017[15,92]	100%	100%
Pergament et al., 2014[16]	100%	100%
Quezada et al., 2015[17]	64.3% (42.3%-81.6%)	99.96 (99.77%-99.99%)

Abbreviation. CI: confidence interval.

T13

In general obstetric populations, screening with cfDNA had a median sensitivity of 100% (range, 40.0%-100%) and a median specificity of 99.94% (range, 99.84%-100%) () for T13.



Abbreviations. CI: confidence interval; FN: false negative; FP: false positive; T13: trisomy 13; TN: true negative; TP: true positive.

Figure 5. Sensitivity and Specificity of cfDNA Tests for T13

For T13, the PPVs ranged from 25.0% to 100% (median, 50.0%) and NPVs from 99.9% to 100% (median, 100%) (

Table 7).

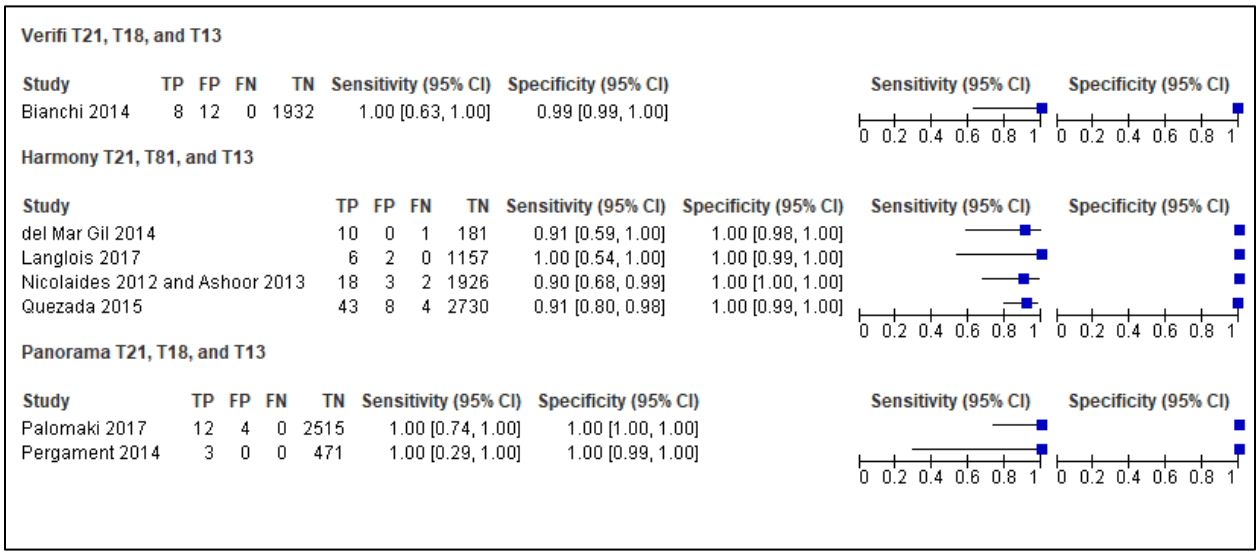
Table 7. Positive and Negative Predictive Values of cfDNA for T13

Study ID	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Ashoor et al., 2013[9]	88.9% (52.4%-98.3%)	99.9% (99.64%-99.97%)
Bianchi et al., 2014[10]	25.0% (9.7%-50.8%)	100%
del Mar Gil et al., 2014[11]	100%	100%
Langlois et al., 2017[12]	No cases of T13 occurred	100%
Norton et al., 2015[14]	50.0% (20.0%-80.0%)	100%
Palomaki et al., 2017[15,92]	50.0% (20.0%-80.0%)	100%
Pergament et al., 2014[16]	No cases of T13 occurred	100%
Quezada et al., 2015[17]	50.0% (14.8%-85.2%)	99.89% (99.78%-99.95%)

Abbreviation. CI: confidence interval.

Trisomies 21, 18, and 13

In general obstetric populations, screening with cfDNA had a median sensitivity of 100% (range, 90.0%-100%) and a median specificity of 99.8% (range, 99.4%-100%) () for the 3 trisomies T21, T18, and T13 taken together.



Abbreviations. CI: confidence interval; FN: false negative; FP: false positive; T13: trisomy 13; T18: trisomy 18; T21: trisomy 21; TN: true negative; TP: true positive.

Figure 6. Sensitivity and Specificity of cfDNA Tests for T21, T18, and T13

For the 3 trisomies T21, T18, and T13 taken together, the PPVs ranged from 40.0% to 100% (median, 84.3%) and NPVs ranged from 99.5% to 100% (median, 100%) (

Table 8).

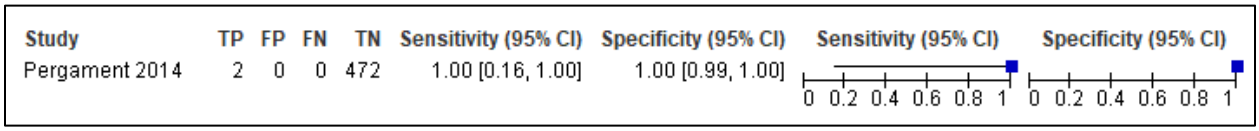
Table 8. Positive and Negative Predictive Values of cfDNA for T21, T18, and T13

Study ID	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Bianchi et al., 2014[10]	40.0% (27.5%-54.0%)	100%
del Mar Gil et al., 2014[11]	100%	99.5% (96.5%-99.9%)
Langlois et al., 2017[12]	75.0% (42.9%-92.3%)	100%
Nicolaides et al., 2012[13]Ashoor et al., 2013[9]	85.7% (65.7%-94.9%)	99.9% (99.6%-99.97%)
Palomaki et al., 2017[15,92]	75.0% (53.0%-88.8%)	100%
Pergament et al., 2014[16]	100%	100%
Quezada et al., 2015[17]	84.3% (72.8%-91.5%)	99.9% (99.6%-99.9%)

Abbreviation. CI: confidence interval.

Sex Chromosome Abnormalities (SCAs)

In a general obstetric population, screening with cfDNA had a sensitivity of 100% and a specificity of 100% (), with a PPV and NPV of 100% each for the 45,X sex chromosome aneuploidy. The performance of cfDNA screening for 45,X was only reported in 1 study and other sex chromosome aneuploidies were not reported in the included studies.



Abbreviations. CI: confidence interval; FN: false negative; FP: false positive; TN: true negative; TP: true positive.

Figure 7. Sensitivity and Specificity of cfDNA Tests for 45,X

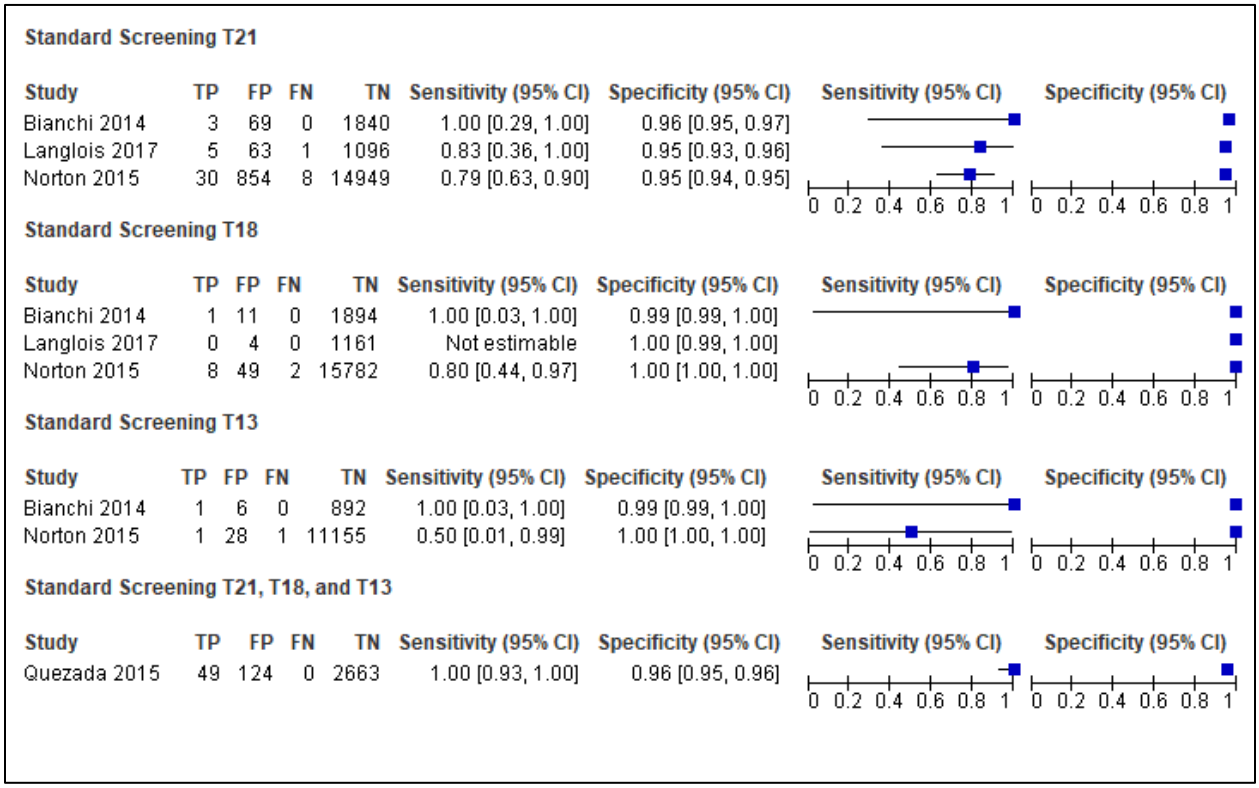
Test Performance of Conventional Screening

Of the 9 included studies that evaluated screening with cfDNA, 4 also evaluated the performance of standard screening.[10,12,14,17] Standard screening comprised maternal serum markers and nuchal translucency.[10,12,14,17]

In general obstetric populations, conventional screening had:

- For T21, a median sensitivity of 83.3% (range, 79.0%-100%) and a median specificity of 94.6% (range, 94.6%-96.4%) (). PPVs ranged from 4.2% to 7.4% and NPVs ranged from 99.9% to 100% (Table 9).
- For T18, a median sensitivity of 90.0% (range, 80.0%-100%) and a median specificity of 99.7% (range, 99.4 %-99.7%) (). PPVs ranged from 8.3% to 14.0% and NPVs ranged from 99.99% to 100% (Table 9).
- For T13, a median sensitivity of 75.0% (range, 50.0%-100%) and a median specificity of 99.5% (range, 99.3 %-99.8%) for T13 (). PPVs ranged from 3.5% to 14.3% and NPVs ranged from 99.99% to 100% (Table 9).

For the 3 trisomies combined, the sensitivity and specificity were 100% and 95.6%, respectively, with a PPV of 28.3% and an NPV of 100% (). No studies reported on the performance of standard screening for sex chromosome aneuploidies.



Abbreviations. CI: confidence interval; FN: false negative; FP: false positive; T13: trisomy 13; T18: trisomy 18; T21: trisomy 21; TN: true negative; TP: true positive.

Figure 8. Sensitivity and Specificity of Standard Screening for T21, T18, and T13

Table 9. Positive and Negative Predictive Values of Standard Screening

Study ID	Condition	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Bianchi et al., 2014[10]	T21	4.2% (3.3%-5.2%)	100%
	T18	8.3% (4.8%-14.1%)	100%
	T13	14.3% (7.0%-27.0%)	100%
Langlois et al., 2017[12]	T21	7.4% (4.9%-10.9%)	99.91 % (99.46%-99.98%)
	T18	No cases of T18 occurred	100%
Norton et al., 2015[14]	T21	3.4% (2.9%-4.0%)	99.95 % (99.90%-99.97%)
	T18	14.0% (9.7%-19.9%)	99.99% (99.96%-100%)
	T13	3.5% (0.84%-13.0%)	99.99 % (99.96%-100%)
Quezada et al., 2015[17]	All trisomies (T21, T18 and T13)	28.3% (25.0%-31.9%)	100%

Abbreviations. CI: confidence interval; T13: trisomy 13; T18: trisomy 18; T21: trisomy 21.

Test Failures

In general, the rates of cfDNA test failures were higher across all 10 studies (range, 0.9%-8.5%; median, 2.8%) than for the failure rate for conventional screening (0.9%), which was reported in only 1 study.[8-17] The 2 highest rates of test failure were observed in a cohort of women at mixed risk (rate of test failure, 8.5%)[16] and in women with twin pregnancies (rate of test failure, 7.2%).[11] Reasons for cfDNA test failure were technical failures of the assay,[9-11,13,14,17] low fetal fraction,[11-14,17] and high variance in cfDNA count.[12,14]

Of the studies that reported outcomes for women who experienced cfDNA test failures:

- One study reported cfDNA test failures for 6 women at both the first and second blood draws.[12] A second-trimester ultrasound identified major structural anomalies in 3 pregnancies and a diagnosis of triploidy was made in all 3 cases.[12] The 3 other women had a negative standard screen and normal second-trimester ultrasound and decided against a third draw.[12] All 3 pregnancies had a normal outcome.[12]
- In the study by Norton et al.,[14] cfDNA testing produced no results for 488 women. In this group, there were 13 aneuploidies: 3 with T21, 1 with T18, 2 with T13, 4 with triploidy, 1 with trisomy 16 mosaic, 1 with deletion 11p, and 1 with a structurally abnormal chromosome.[14] Standard screening detected the 6 common aneuploidies where there was no cfDNA result.[14]
- In the study by Quezada et al.,[17] cfDNA testing produced no results for 54 pregnancies. In this group, there were 49 non-trisomic cases, 2 cases of T21, and 3 cases of miscarriage with no karyotype.[17]
- In the study by Palomaki et al.,[15] 9 women had a positive serum screen after a failed cfDNA test and 8 of these had normal birth outcomes.[15] The ninth woman was diagnosed with a mosaic condition herself after a positive cfDNA test from another sequencing laboratory and delivered a normal female infant.[15]

Invasive Testing

Screening using cfDNA reduced the use of invasive testing in 1 RCT.[8] Of the 17 women assessed as being at high risk of a T21 pregnancy using conventional FTS, 35.3% (6 of 17) opted for invasive testing, 52.9% (9 of 17) opted for additional cfDNA testing, and 11.8% (2 of 17) opted for no further evaluation.[8] A further 6 women assessed as being at low risk of a T21 pregnancy using conventional FTS opted for invasive testing.[8] In the cfDNA plus ultrasound group, 2 women assessed as low risk opted for invasive testing for personal reasons.[8] Overall, 1.7% (12 of 688) of the women in the FTS group and 0.3% (2 of 688) of the women in the cfDNA plus ultrasound group opted for invasive testing.[8]

Of the 9 test accuracy studies, 3 reported the use of invasive testing after a positive screen with cfDNA or conventional screening:

- In the study by Bianchi et al.,[10] 17 women with positive results by standard screening chose to undergo invasive prenatal procedures compared with 27 women with negative results (chorionic villus sampling [CVS], 5; amniocentesis, 22).[10] All fetal karyotypes were normal and the results of cfDNA testing were negative for T21, T18, and T13.[10] Bianchi et al.[10] estimated that if all pregnant women had undergone cfDNA testing as a primary screening method and if all women with positive results had undergone post-test counseling and had decided to undergo an

invasive procedure, there would have been a relative reduction of 89% in the number of diagnostic invasive procedures required to confirm a positive screening result.[10]

- In the study by Langlois et al.,[12] the total invasive diagnostic procedure rate was 2% (95% CI, 1.3%-3%), but the rate was estimated to be as high as 6.8% (95% CI, 5.4%-8.4%) based on traditional screening and ultrasound examination without cfDNA analysis.[12] The rate of invasive diagnostic testing in cfDNA-negative women was 1.2% (95% CI, 0.7%-2%).[12] For the 2 women whose pregnancies were cfDNA positive for T18 and T13 and who underwent amniocentesis, the fetuses were found to have normal karyotypes; both births were live and normal.[12] Overall, 59 women with a positive traditional screen chose to avoid amniocentesis based on a negative cfDNA screen.[12] All pregnancies had normal outcomes. Langlois et al.[12] estimated that if cfDNA had been conducted as the only primary screen, up to 62 amniocenteses would have been avoided.
- In the study by Palomaki et al.,[15] all 16 women with positive cfDNA screens proceeded to invasive testing and diagnostic testing. Testing confirmed 9 cases and 7 of these pregnancies were terminated.[15]

Key Question 3

Maternal Age

No studies compared outcomes or test performance by maternal age.

Maternal Weight

Greater maternal weight appeared to be associated with higher rates of cfDNA test failures:

- In the study by Langlois et al.,[12] 11 women had a test failure at the first blood draw. Excluding the 3 cases where the low-fetal fraction was due to triploidy, the maternal weight of the remaining 8 women with a failed cfDNA test was greater than 70 kg in 6 of them, with a mean maternal weight of 94 kg (range, 58.5-131 kg).[12]
- The median maternal weight was 93.7 kg in women with a low fetal fraction vs. 65.8 kg in women with a successful cfDNA test ($P < .001$).[14]
- cfDNA test failures were strongly associated with a maternal weight of 80 kg or higher (risk ratio, 11.4; 95% CI, 6.3-21; $P < .001$).[15]

Singleton or Multifetal Pregnancies

Only 2 studies included twin pregnancies,[11,15] but direct comparisons of outcomes or test performance were not conducted for singleton and multifetal pregnancies.

Gestational Age

In 1 study, a slightly higher rate of test failures in low-risk women compared with high-risk women (rate: 8.5% vs. 8.1%; $P = 0.86$) was attributed to a lower gestational age in the low-risk cohort compared with the overall cohort (a median of 12.9 weeks vs. 14.3 weeks; no formal statistical testing was reported).[16]

Presence of Aneuploidies

In 1 study, the prevalence of aneuploidies was lower in women with a successful cfDNA test compared with women with a failed cfDNA test (rate: 0.4% vs. 2.7%; $P < .001$).[14] The prevalence of aneuploidies in women with a low fetal fraction was 4.7%.[14]

Key Question 4

Study Characteristics

We found 8 economic studies, published in the last 5 years, that evaluated the costs and benefits of cfDNA screening in the U.S. (Table 10 and Appendix C, Table C7).[18-25] We rated the risk of bias in these studies as follows:

- Two studies were rated low risk of bias[24,25] with only minor methodological concerns.
- Four studies were rated moderate risk of bias[18,20-22] due to concerns about model assumptions and design (e.g., a lack of a complete model diagram, time horizons not being stated explicitly) and sensitivity analyses.
- Two studies were rated high risk of bias[19,23] due to substantial concerns about limited reporting on the models used, a lack of clarity on the time horizon used, and limited use of sensitivity analysis.

Table 10. Study Characteristics of Eligible Economic Studies Evaluating cfDNA Screening

Study ID Study Risk of Bias	Population	Conditions	Economic Analytic Method
Benn et al., 2015[18] Moderate	Theoretical cohort of 3,952,841 live births, representing the U.S. general obstetric prenatal screening population in 2012	T21, T18, T13, and 45,X (Turner syndrome)	Cost impact
Crimmins et al., 2017[19] High	Pregnant women choosing aneuploidy risk assessment, who presented for care between 15 and 21 weeks at a single urban center	T21	Cost impact
Evans et al., 2015[20] Moderate	Theoretical cohort of 1,000,000 pregnant women	T21	Cost impact
Fairbrother et al., 2016[21] Moderate	Theoretical cohort of 4,000,000 pregnant women, representative of the U.S. general obstetric prenatal screening population in 1 year	T21, T18, and T13	Cost impact
Kaimal et al., 2015[22] Moderate	Theoretical cohort of pregnant women desiring prenatal testing (screening or diagnostic or both)	T21, T18, T13, sex chromosome aneuploidy (45,X; 47,XXX; 47,XXY; 47,XYY), a pathogenic copy number variant (microdeletion or duplication) or other rare chromosomal abnormality, or a variant of uncertain significance	Cost effectiveness
Shiv et al., 2017[23] High	Theoretical cohort of 3,000 pregnant women	T21 and all detectable aneuploidies	Cost impact
Walker et al., 2015[24] Low	Theoretical cohort of 1,000,000 pregnant women at 10 weeks' gestation	T21	Cost effectiveness
Walker et al., 2015[25]	Theoretical cohort of 1,000,000 pregnant women	T21, T18, and T13	Cost effectiveness

Low	representative of the U.S. general obstetric prenatal screening population		

Abbreviations. 45,X: Turner syndrome; 47,XXX: Triple X syndrome; 47,XXY: Klinefelter syndrome; 47,XYY: Jacob's syndrome; T13: trisomy 13; T18: trisomy 18; T21: trisomy 21.

Six of the 8 studies[18,20-22,24,25] were based on theoretical cohorts that were representative of the general obstetric population and 2 studies[19,23] were based on data and assumptions for populations from single centers.

Three of the 8 studies[18,23,24] compared conventional maternal serum screening plus ultrasound in the first and second trimester with universal cfDNA alone. Fairbrother et al.[21] compared FTS plus ultrasound with cfDNA plus ultrasound. Crimmins et al.[19] compared second-trimester QUAD screening plus ultrasound with cfDNA plus ultrasound among women who did not undergo FTS. Walker et al.[25] compared conventional maternal serum screening plus ultrasound in the first and second trimesters with universal plus contingent cfDNA screening. Evans et al.[20] compared conventional screening with a range of screening strategies, including primary cfDNA screening and contingent cfDNA screening in the first trimester. Kaimal et al.[22] compared chromosomal microarray, multiple marker screening, cfDNA screening, and nuchal translucency screening alone, in combination, or in sequence.

Three of the 8 studies evaluated the costs and pregnancy outcomes of screening for T21 alone;[19,20,24] 2 for trisomies 21, 18, and 13,[21,25] 1 for trisomies 21, 18, and 13 and 45,X,[18] 1 for T21 and all detectable aneuploidies,[23] and 1 for trisomies 21, 18, and 13, sex chromosome aneuploidy (45,X 47,XXX; 47,XXY; 47,XYY), a pathogenic copy number variant (microdeletion or duplication) or other rare chromosomal abnormality, or a variant of uncertain significance.[22]

Study Findings

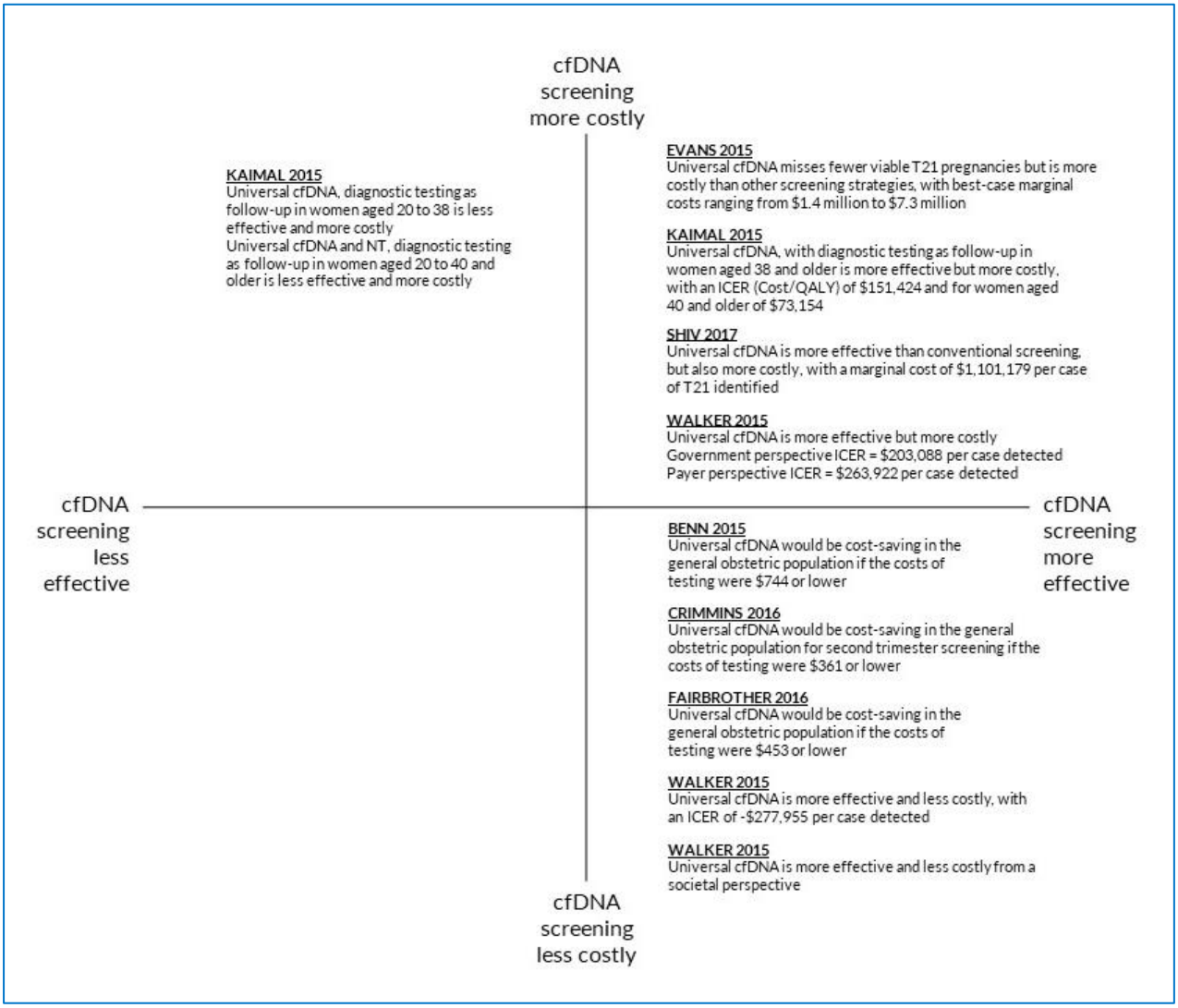
Cost-Effectiveness

Universal cfDNA testing varied in its estimated effectiveness and value for money (Figure 9). Universal cfDNA screening was more effective and less costly than other screening strategies in 4 studies.[18,19,21,24] The study by Walker et al.,[24] which had a low risk of bias, found that cfDNA screening at a cost of $400 was more effective and less costly than conventional screening. In 3 other studies, 2 at moderate risk of bias and 1 at high risk of bias, universal cfDNA screening was more effective than conventional screening in the first or second trimesters with cfDNA screening costs of $744, $361, or $453 and lower.[18,19,21]

Universal cfDNA screening was also more effective but more costly than other screening strategies in 2 studies.[20,23] In 1 study with a high risk of bias,[23] universal cfDNA screening was more effective than conventional screening but also more costly, with a marginal cost of $1,101,179 per case of T21 identified. In 1 study with a moderate risk of bias,[20] universal cfDNA screening was more effective but more costly than other screening strategies, even in the best-case scenario using model assumptions that were most favorable to primary cfDNA screening.

Two studies found that effectiveness and costs varied depending on the population being screened and the economic perspective taken.[22,25] The study by Kaimal et al.,[22] with a moderate risk of bias, found that cfDNA screening was more costly than other strategies for women of any age, but was only more effective in women aged 38 and older. From a government or payer perspective, Walker et al.[25] found

that cfDNA screening was more effective but more costly than other screening strategies. From the societal perspective, universal cfDNA screening remained more effective, but was also potentially less costly than other screening strategies.[25] We assessed this study as having a low risk of bias.[25]



Source. Adapted from Nshimyumukiza et al., 2018.[68]

Figure 9. Cost-Effectiveness Plane for Eligible Economic Studies

Pregnancy Outcomes

The included economic studies also modelled the pregnancy outcomes of cfDNA screening.

Aneuploidy Cases Detected

Universal cfDNA screening increased the number of aneuploidy cases detected in 5 studies:

- In the study by Benn et al.,[18] 12.4% more cases of T21, T18, T13 and 45,X were detected with cfDNA screening (12,717 cases) compared with conventional screening (11,314 cases).[18] This study was assessed as having a moderate risk of bias.
- Fairbrother et al.,[21] reported that 15.3% more cases of T21, T18, and T13 were detected with cfDNA screening (8,993 cases) compared with conventional screening (7,799 cases).[21] This study was assessed as having a moderate risk of bias.
- In the study by Kaimal et al.,[22] cfDNA screening alone or with nuchal translucency measurement identified more cases of T21 in women of all ages than all other screening strategies except for the concurrent use of cfDNA and maternal serum screening.[22] For example, in 1,000 women aged 20 to 29, cfDNA screening identified 79 cases of T21 or 80 cases when nuchal translucency measurement was added. In contrast, maternal serum screening identified only 65 cases or 80 cases with concurrent cfDNA screening.[22] This study was assessed as having a moderate risk of bias.
- In the Walker et al.[24] study, cfDNA screening detected 29.9% more cases of T21 (1,915 cases) than conventional integrated screening (1,474 cases). Of these, 29.9% more cases were diagnosed in the cfDNA screening group (1,360 cases) than in the conventional screening group (1,047 cases).[24] This study was assessed as having a low risk of bias.
- In the second study by Walker et al.,[25] 35.5% more cases of T21, T18, and T13 were detected with universal cfDNA screening (3,409 cases) than with conventional screening (2,516 cases).[25] This study was assessed as having a low risk of bias.

In the study by Evans et al.,[20] fewer cases of T21 were missed with cfDNA screening (12 of 1,000,000 screens) than with conventional screening strategies (61 of 1,000,000 to 273 of 1,000,000 depending on the specific conventional screening comparator strategy). This study was assessed as having a moderate risk of bias.

Shiv et al.[23] found that universal cfDNA screening detected 1 more case of T21 than conventional screening and missed 0.05 cases compared with 0.25 cases in a cohort of 3,000 women.[23] For all aneuploidies, conventional screening detected 1 more case of any detectable aneuploidy and missed 1 fewer case compared with universal cfDNA screening.[23] This study was assessed as having a high risk of bias.

None of the studies reported formal statistical testing of differences between screening strategies, but most of the studies explored the robustness of the results with sensitivity analyses.

Affected Births

Benn et al.[18] found that universal cfDNA reduced the number of infants born with T21, T18, T13 and 45,X by 33.4% (4,842 affected births averted) compared with conventional screening (3,629 affected births averted). This study was assessed as having a moderate risk of bias. Another study by Walker et al.[24] showed similar results, with 14.9% fewer liveborn infants with T21 with cfDNA screening (1,039 births) than with conventional screening (1,221 births).[24] This study was assessed as having a low risk of bias.

Use of Invasive Tests

Universal cfDNA screening reduced the number of invasive diagnostic tests undertaken and procedure-related losses in 5 studies:

- In the study by Benn et al.,[18] 60.0% fewer invasive tests were undertaken with cfDNA screening (24,596 tests) than with conventional screening (61,430 tests), with an associated reduction in procedure-related losses of 73.5% (procedure-related losses: 70 with cfDNA screening vs. 264 with conventional screening).[18] This study was assessed as having a moderate risk of bias.
- Fairbrother et al.,[21] reported that 88.3% fewer invasive tests were undertaken with cfDNA screening (17,303 tests) than with conventional screening (147,311 tests).[21] Of these, 8,342 were unnecessary with cfDNA screening and 139,540 with conventional screening, for a reduction of 94.0% in unnecessary tests with cfDNA screening.[21] The number of procedure-related losses was also 94% lower with cfDNA screening (42 losses) than with conventional screening (698 losses).[21] This study was assessed as having a moderate risk of bias.
- Shiv et al.[23] found that universal cfDNA resulted in 101 invasive tests avoided for T21 and 59 for any detectable aneuploidy compared with conventional screening.[23] This study was assessed as having a high risk of bias.
- In the study by Walker et al.,[24] 94.3% fewer unnecessary invasive tests were undertaken with cfDNA screening (687 tests) than with conventional screening (11,972 tests), with an associated reduction of 94.5% in procedure-related losses (5 with cfDNA screening vs. 91 with conventional screening).[24] This study was assessed as having a low risk of bias.

However, not all cfDNA studies found a reduction in invasive testing. In 1 study,[22] cfDNA screening, alone or with nuchal translucency measurement, increased the numbers of diagnostic procedures and procedure-related losses.[22] In 1,000 women aged 20 to 29, the number of diagnostic procedures was 7,509 for cfDNA screening alone and 9,498 for cfDNA with nuchal translucency measurement[22] but only 7,073 for the maternal serum screen alone (procedures per case diagnosed: 20.9 cfDNA alone, 13.1 cfDNA with nuchal translucency, 5.4 maternal serum screen alone).[22] The numbers of procedure-related losses were 18 for cfDNA screening alone and 24 for cfDNA with nuchal translucency measurement, compared with 18 for the maternal serum screen alone.[22] This study was assessed as having a moderate risk of bias.

For women who presented in the second trimester, Crimmins et al. found that 55.4% fewer invasive tests were undertaken with cfDNA screening than with QUAD screening, with an associated reduction in the rate of procedure-related losses of 57% (from 65 losses with QUAD to 28 with cfDNA).[19] This study was assessed as having a high risk of bias.

Genetic Counseling

Although cfDNA screening is usually undertaken during the first trimester, 1 study found that screening with cfDNA during the second trimester reduced the number of patients requiring genetic counseling by 78% (rate of genetic counseling: 2.9% with cfDNA screening vs. 14.7% with QUAD screening).[19] This study was assessed as having a high risk of bias. We did not find any studies that reported the impact of cfDNA screening on conventional FTS.

Summary

Effectiveness and Harms

In summary (Table 11), cfDNA screening:

- Had a lower FP screening rate than conventional FTS (0% vs. 2.5%; *P* value not reported) (low-quality evidence, based on 1 RCT)
- Had a test failure rate ranging from 0.9% to 8.5% (very-low-quality evidence, based on 1 RCT, 8 cohort studies, and 1 case-control study)
- Resulted in lower rates of invasive testing than conventional screening (low-quality evidence, based on 1 RCT, and very-low-quality evidence from 2 cohort studies)

Table 11. GRADE Summary of Evidence: Effectiveness and Harms

Number of Participants (N) Studies (k)	Findings	Certainty of Evidence	Rationale
Outcome: FP Rate for T21			
N = 1,400 1 RCT[8]	cfDNA testing had a lower FP screening rate than conventional FTS (0% vs. 2.5%; *P* value not reported).	⨁⨁◯◯ LOW	Downgraded 1 level each for risk of bias and imprecision (i.e., wide CIs)
Outcome: Test Failures			
N = 30,238 1 RCT, 8 cohort studies, and 1 case-control[8-17]	cfDNA test failure rates ranged from 0.9% to 8.5%. The highest rates of failures occurred in studies with twin pregnancies only or with a mixed risk population.	⨁◯◯◯ VERY LOW	Downgraded 1 level each for risk of bias, inconsistency, and imprecision (i.e., not assessable)[a]
Outcome: Invasive Testing			
N = 1,400 1 RCT[8]	Overall, 1.7% (12 of 688) of women in the FTS group and 0.3% (2 of 688) in the cfDNA plus ultrasound group opted for invasive testing.	⨁⨁◯◯ LOW	Downgraded 1 level each for risk of bias and imprecision (i.e., not assessable)
N = 3,117 2 cohort studies[10,12]	cfDNA screening was associated with lower rates of invasive testing.	⨁◯◯◯ VERY LOW	Downgraded 1 level each for risk of bias, indirectness (author estimates, not observed effects), and imprecision (i.e., not assessable)

Abbreviations. cfDNA: cell-free DNA; CI: confidence interval; FP: false positive; FTS: first-trimester screening; RCT: randomized controlled trial; T21: trisomy 21. Note. [a]For test failure rates, we combined information from the RCT, cohort studies, and the case-control study. The certainty of evidence started as low.

Test Performance

For the common trisomies (T21, T18, and T13):

- 6 of 1,000 pregnancies would be expected to be affected
- cfDNA screening would be expected to miss no cases (moderate-quality evidence from 6 studies,

- Table 12) and up to 6 of 1,000 unaffected pregnant women would undergo ultimately unnecessary invasive testing (moderate-quality evidence from 6 studies,
- Table 12)
- Conventional screening would be expected to miss up to 1 case in 1,000, assuming the same prevalence of the common trisomies (moderate-quality evidence from 1 study,
- Table 14), and 44 in 1,000 women with unaffected pregnancies (range, 37-52) would undergo unnecessary invasive testing (moderate-quality evidence from 1 study,
- Table 14)
- The median PPV for cfDNA was 79.7% (range, 40.0%-100%) (very-low-quality evidence from 6 studies, Table 13) compared with 28.3% (95% CI, 25.0%-31.9%) for conventional screening (moderate-quality evidence from 1 study,

- Table 15)

For T21:

- 3 of 1,000 pregnancies would be expected to be affected
- cfDNA screening would be expected to miss no cases (moderate-quality evidence from 7 studies,
- Table 16) and up to 3 unaffected pregnant women would undergo ultimately unnecessary invasive testing (moderate-quality evidence from 7 studies,
- Table 16)
- Conventional screening would be expected to miss up to 1 case, assuming the same prevalence of T21 (very-low-quality evidence from 3 studies,
- Table 18), and from 36 to 54 unaffected pregnant women would undergo unnecessary invasive testing (moderate-quality evidence from 3 studies,
- Table 18)
- The median PPV for cfDNA was 97.0% (range, 45.5%-100%) (very-low-quality evidence from 7 studies,

- Table 17) compared with 4.2% (range, 3.4%-7.4%) for conventional screening (moderate-quality evidence from 3 studies, Table 19)

For T18:

- 1 of 1,000 pregnancies would be expected to be affected
- cfDNA screening would be expected to miss no cases (moderate-quality evidence from 7 studies,
- Table 20) and up to 2 unaffected pregnant women would undergo ultimately unnecessary invasive testing (moderate-quality evidence from 7 studies,
- Table 20)
- Conventional screening would be expected to miss no cases, assuming the same prevalence of T18 (very-low-quality evidence from 3 studies,
- Table 22), and from 3 to 6 unaffected pregnant women would undergo unnecessary invasive testing (moderate-quality evidence from 3 studies,
- Table 22)
- The median PPV for cfDNA was 77.1% (range, 45.5%-100%) (very-low-quality evidence from 7 studies,

- Table 21) compared with 8.3% (range, 0%-14.0%) for conventional screening (moderate-quality evidence from 3 studies, Table 23)

For T13:

- Up to 1 of 1,000 pregnancies would be expected to be affected
- cfDNA screening would be expected to miss up to 1 case (low-quality evidence from 7 studies,
- Table 24) and up to 2 unaffected pregnant women would undergo ultimately unnecessary invasive testing (moderate-quality evidence from 7 studies,
- Table 24)
- Conventional screening would be expected to miss up to 1 case, assuming the same prevalence of T13 (very-low-quality evidence from 2 studies,
- Table 26), and from 3 to 4 unaffected pregnant women would undergo unnecessary invasive testing (moderate-quality evidence from 2 studies,
- Table 26)
- The median PPV for cfDNA was 50.0% (range, 25.0%-88.9%) (very-low-quality evidence from 7 studies, Table 25) compared with 3.5% and 14.3% for conventional screening (low-quality evidence from 2 studies, Table 27)

For the common trisomies (T21, T18, and T13) in twin pregnancies:

- 52 of 1,000 pregnancies would be expected to be affected
- cfDNA screening would be expected to miss 5 cases (from none to 23) (low-quality evidence from 1 study, Table 28) and no unaffected pregnant women (from none to 19) would undergo ultimately unnecessary invasive testing (moderate-quality evidence from 1 study, Table 28)
- The PPV for cfDNA was 100% (moderate-quality evidence from 1 study, Table 29)

For the sex chromosome aneuploidies:

- 4 of 1,000 pregnancies would be expected to be affected
- cfDNA screening would be expected to miss no cases (from none to 3) (very-low-quality evidence from 1 study, Table 30) and no unaffected pregnant women (from none to 8) would undergo ultimately unnecessary invasive testing (very-low-quality evidence from 1 study, Table 30)
- The PPV for cfDNA was 100% (low-quality evidence from 1 study,
- Table 31)

Table 12. GRADE Summary of Evidence: Test Accuracy of cfDNA Tests for All Common Trisomies (T21, T18, and T13)

Test Results	Number of Results per 1,000 Patients Tested (Range)			Number of Participants and Studies	Certainty of Evidence (GRADE)	Rationale
	Prevalence 0.41% Seen in the Study with the Lowest Prevalence	Prevalence 0.57% Seen in the Study with the Median Prevalence	Prevalence 1.69% Seen in the Study with the Highest Prevalence			
True positives	4 to 4	5 to 6	15 to 17	10,856 participants, 6 studies[9,10,12,13,15-17]	⊕⊕⊕◯ MODERATE	Downgraded 1 level for risk of bias
False negatives	0 to 0	0 to 1	0 to 2			
True negatives	990 to 996	988 to 994	977 to 983	10,856 participants, 6 studies[9,10,12,13,15-17]	⊕⊕⊕◯ MODERATE	Downgraded 1 level for risk of bias
False positives	0 to 6	0 to 6	0 to 6			

Note. Range of sensitivities: 0.91 to 1.00; range of specificities: 0.99 to 1.00.

Table 13. GRADE Summary of Evidence: Test Performance (PPV and NPV) of cfDNA Tests for All Common Trisomies (T21, T18, and T13)

Outcome	Number of Participants and Studies	Median (Range)	Test Accuracy CoE	Rationale
PPV	10,856 participants, 6 studies[9,10,12,13,15-17]	79.7% (40.0% to 100%)	⊕◯◯◯ VERY LOW	Downgraded 1 level each for risk of bias, inconsistency (i.e., different results across studies), and imprecision (i.e., wide CIs)
NPV	10,856 participants, 6 studies[9,10,12,13,15-17]	100% (99.9% to 100%)	⊕⊕⊕◯ MODERATE	Downgraded 1 level for risk of bias

Abbreviations. CI: confidence interval; CoE: certainty of evidence; NPV: negative predictive value; PPV: positive predictive value.

Table 14. GRADE Summary of Evidence: Test Accuracy of Conventional Screening for All Common Trisomies (T21, T18, and T13)

Test Results	Number of Results per 1,000 Patients Tested (95% CI)		Number of Participants and Studies	Certainty of Evidence (GRADE)	Rationale
	Prevalence 1.73% Seen in this study	Prevalence 0.57% Median from the cfDNA studies			
True positives	17 (16 to 17)	6 (5 to 6)	2,836 participants, 1 study[17]	⨁⨁⨁◯ MODERATE	Downgraded 1 level for risk of bias
False negatives	0 (0 to 1)	0 (0 to 1)			
True negatives	939 (931 to 946)	950 (942 to 957)	2,836 participants, 1 study[17]	⨁⨁⨁◯ MODERATE	Downgraded 1 level for risk of bias
False positives	44 (37 to 52)	44 (37 to 52)			

Abbreviation. CI: confidence interval. Note. Single study sensitivity: 1.00 (95% CI, 0.93 to 1.00); single study specificity: 0.96 (95% CI, 0.95 to 0.96).

Table 15. GRADE Summary of Evidence: Test Performance (PPV and NPV) of Conventional Screening for All Common Trisomies (T21, T18, and T13)

Outcome	Number of Participants and Studies	Effect (95% CI)	Test Accuracy CoE	Rationale
PPV	2,836 participants, 1 study[17]	28.3% (25.0% to 31.9%)	⊕⊕⊕◯ MODERATE	Downgraded 1 level for risk of bias
NPV	2,836 participants, 1 study[17]	100% (NA)	⊕⊕⊕◯ MODERATE	Downgraded 1 level for risk of bias

Abbreviations. CI: confidence interval; CoE: certainty of evidence; NPV: negative predictive value; PPV: positive predictive value.

Table 16. GRADE Summary of Evidence: Test Accuracy of cfDNA Tests for T21

Test Results	Number of Results per 1,000 Patients Tested (Range)			Number of Participants and Studies	Certainty of Evidence (GRADE)	Rationale
	Prevalence 0.21% Seen in the Study with the Lowest Prevalence	Prevalence 0.28% Seen in the Study with the Median Prevalence	Prevalence 1.15% Seen in the Study with the Highest Prevalence			
True positives	2 to 2	3 to 3	11 to 12	26,697 participants, 7 studies[10,12-17]	⊕⊕⊕◯ **MODERATE**	Downgraded 1 level for risk of bias
False negatives	0 to 0	0 to 0	-1 to 1			
True negatives	995 to 998	994 to 997	985 to 989	26,697 participants, 7 studies[10,12-17]	⊕⊕⊕◯ **MODERATE**	Downgraded 1 level for risk of bias
False positives	0 to 3	0 to 3	-1 to 4			

Note. Range of sensitivities: 1.00 to 1.00; range of specificities: 1.00 to 1.00.

Table 17. GRADE Summary of Evidence: Test Performance (PPV and NPV) of cfDNA Tests for T21

Outcome	Number of Participants and Studies	Median (Range)	Test Accuracy CoE	Rationale
PPV	26,697 participants, 7 studies[10,12-17]	97.0% (45.5% to 100%)	⊕◯◯◯ VERY LOW	Downgraded 1 level each for risk of bias, inconsistency (i.e., different results across studies), and imprecision (i.e., wide CIs)
NPV	26,697 participants, 7 studies[10,12-17]	100% (all 100%)	⊕⊕⊕◯ MODERATE	Downgraded 1 level for risk of bias

Abbreviations. CI: confidence interval; CoE: certainty of evidence; NPV: negative predictive value; PPV: positive predictive value.

Table 18. GRADE Summary of Evidence: Test Accuracy of Conventional Screening for T21

Test Results	Number of Results per 1,000 Patients Tested (Range)				Number of Participants and Studies	Certainty of Evidence (GRADE)	Rationale
	Prevalence 0.16% Seen in the Study with the Lowest Prevalence	Prevalence 0.24% Seen in the Study with the Median Prevalence	Prevalence 0.52% Seen in the Study with the Highest Prevalence	Prevalence 0.28% Median from the cfDNA Studies			
True positives	1 to 2	2 to 2	4 to 5	2 to 3	18,918 participants,[1,0,12,14] 3 studies	⊕◯◯◯ VERY LOW	Downgraded 1 level each for risk of bias, inconsistency (i.e., different results across studies), and imprecision (i.e., wide CIs)
False negatives	0 to 1	0 to 0	0 to 1	0 to 1			
True negatives	944 to 962	943 to 962	941 to 959	943 to 961	18,918 participants,[1,0,12,14] 3 studies	⊕⊕⊕◯ **MODERATE**	Downgraded 1 level for risk of bias
False positives	36 to 54	36 to 55	36 to 54	36 to 54			

Abbreviation. CI: confidence interval. Note. Range of sensitivities: 0.79 to 1.00; range of specificities: 0.95 to 0.96.

Table 19. GRADE Summary of Evidence: Test Performance (PPV and NPV) of Conventional Screening for T21

Outcome	Number of Participants and Studies	Median (Range)	Test Accuracy CoE	Rationale
PPV	18,918 participants,[10,12,14] 3 studies	4.2% (3.4% to 7.4%)	⨁⨁⨁◯ MODERATE	Downgraded 1 level for risk of bias
NPV	18,918 participants,[10,12,14] 3 studies	99.95% (99.91% to 100%)	⨁⨁⨁◯ MODERATE	Downgraded 1 level for risk of bias

Abbreviations. CoE: certainty of evidence; NPV: negative predictive value; PPV: positive predictive value.

Table 20. GRADE Summary of Evidence: Test Accuracy of cfDNA Tests for T18

Test Results	Number of Results per 1,000 Patients Tested (Range)			Number of Participants and Studies	Certainty of Evidence (GRADE)	Rationale
	Prevalence 0% Seen in the Study with the Lowest Prevalence	Prevalence 0.1% Seen in the Study with the Median Prevalence	Prevalence 0.42% Seen in the Study with the Highest Prevalence			
True positives	0 to 0	1 to 1	4 to 4	26,697 participants, 7 studies[10,12-17]	⨁⨁⨁◯ **MODERATE**	Downgraded 1 level for risk of bias
False negatives	0 to 0	0 to 0	0 to 0			
True negatives	998 to 1000	997 to 999	994 to 996	26,697 participants, 7 studies[10,12-17]	⨁⨁⨁◯ **MODERATE**	Downgraded 1 level for risk of bias
False positives	0 to 2	0 to 2	0 to 2			

Note. Range of sensitivities: 0.90 to 1.00; range of specificities: 1.00 to 1.00.

Table 21. GRADE Summary of Evidence: Test Performance (PPV and NPV) of cfDNA Tests for T18

Outcome	Number of Participants and Studies	Median (Range)	Test Accuracy CoE	Rationale
PPV	26,697 participants, 7 studies[10,12-17]	77.1% (40.0% to 100%)	⊕◯◯◯ VERY LOW	Downgraded 1 level each for risk of bias, inconsistency (i.e., different results across studies), and imprecision (i.e., wide CIs)
NPV	26,697 participants, 7 studies[10,12-17]	100% (99.96% to 100%)	⊕⊕⊕◯ MODERATE	Downgraded 1 level for risk of bias

Abbreviations. CI: confidence interval; CoE: certainty of evidence; NPV: negative predictive value; PPV: positive predictive value.

Table 22. GRADE Summary of Evidence: Test Accuracy of Conventional Screening for T18

Test Results	Number of Results per 1,000 Patients Tested (Range)				Number of Participants and Studies	Certainty of Evidence (GRADE)	Rationale
	Prevalence 0% Seen in the Study with the Lowest Prevalence	Prevalence 0.05% Seen in the Study with the Median Prevalence	Prevalence 0.06% Seen in the Study with the Highest Prevalence	Prevalence 0.1% Median from the cfDNA Studies			
True positives	0 to 0	0 to 1	0 to 1	1 to 1	18,912 participants,[1 0,12,14] 3 studies	⊕◯◯◯ VERY LOW	Downgraded 1 level each for risk of bias, inconsistency (i.e., different results across studies), and imprecision (i.e., wide CIs)
False negatives	0 to 0	-1 to 1	0 to 1	0 to 0			
True negatives	944 to 997	994 to 996	994 to 996	993 to 996	18,912 participants,[1 0,12,14] 3 studies	⊕⊕⊕◯ **MODERATE**	Downgraded 1 level for risk of bias
False positives	3 to 6	4 to 6	3 to 5	3 to 6			

Abbreviations. cfDNA: cell-free DNA; CI: confidence interval. Note. Range of sensitivities: 0.80 to 1.00; range of specificities: 0.99 to 1.00.

Table 23. GRADE Summary of Evidence: Test Performance (PPV and NPV) of Conventional Screening for T18

Outcome	Number of Participants and Studies	Median (Range)	Test Accuracy CoE	Rationale
PPV	18,918 participants,[10,12,14] 3 studies	8.3% (0% to 14.0%)	⊕⊕◯◯ **LOW**	Downgraded 1 level each for risk of bias and imprecision (i.e., wide CIs)
NPV	18,918 participants,[10,12,14] 3 studies	100% (99.99% to 100%)	⊕⊕⊕◯ MODERATE	Downgraded 1 level for risk of bias

Abbreviations. CI: confidence interval; CoE: certainty of evidence; NPV: negative predictive value; PPV: positive predictive value.

Table 24. GRADE Summary of Evidence: Test Accuracy of cfDNA Tests for T13

Test results	Number of Results per 1,000 Patients Tested (Range)			Number of Participants and Studies	Certainty of Evidence (GRADE)	Rationale
	Prevalence 0% Seen in the Study with the Lowest Prevalence	Prevalence 0.05% Seen in the Study with the Median Prevalence	Prevalence 0.51% Seen in the Study with the Highest Prevalence			
True positives	0 to 0	0 to 1	2 to 5	22,003 participants, 7 studies[9,10,12,14-17]	⊕⊕◯◯ **LOW**	Downgraded 1 level each for risk of bias and inconsistency (i.e., differences in results across studies)
False negatives	0 to 0	-1 to 1	0 to 3			
True negatives	998 to 1000	998 to 1000	993 to 995	22,003 participants, 7 studies[9,10,12,14-17]	⊕⊕⊕◯ **MODERATE**	Downgraded 1 level for risk of bias
False positives	0 to 2	-1 to 2	0 to 2			

Note. Range of sensitivities: 0.40 to 1.00; range of specificities: 1.00 to 1.00.

Table 25. GRADE Summary of Evidence: Test Performance (PPV and NPV) of cfDNA Tests for T13

Outcome	Number of Participants and Studies	Median (Range)	Test Accuracy CoE	Rationale
PPV	22,003 participants, 7 studies[9,10,12,14-17]	50.0% (25.0% to 88.9%)	⊕◯◯◯ VERY LOW	Downgraded 1 level each for risk of bias, inconsistency (i.e., different results across studies), and imprecision (i.e., wide CIs)
NPV	22,003 participants, 7 studies[9,10,12,14-17]	100% (99.89% to 100%)	⊕⊕⊕◯ MODERATE	Downgraded 1 level for risk of bias

Abbreviations. CI: confidence interval; CoE: certainty of evidence; NPV: negative predictive value; PPV: positive predictive value.

Table 26. GRADE Summary of Evidence: Test Accuracy of Conventional Screening for T13

Test Results	Number of Results per 1,000 Patients Tested (Range)			Number of Participants and Studies	Certainty of Evidence (GRADE)	Rationale
	Prevalence 0.02% Seen in the Study with the Lowest Prevalence	Prevalence 0.11% Seen in the Study with the Highest Prevalence	Prevalence 0.05% Median from the cfDNA Studies			
True positives	0 to 0	1 to 1	0 to 1	12,084 participants,[10,14] 2 studies	⊕◯◯◯ VERY LOW	Downgraded 1 level each for risk of bias, inconsistency (i.e., different results across studies), and imprecision (i.e., wide CIs)
False negatives	0 to 0	0 to 0	-1 to 1			
True negatives	993 to 997	992 to 996	993 to 997	12,084 participants,[10,14] 2 studies	⊕⊕⊕◯ **MODERATE**	Downgraded 1 level for risk of bias
False positives	3 to 7	3 to 7	3 to 4			

Abbreviation. CI: confidence interval. Note. Range of sensitivities: 0.50 to 1.00; range of specificities: 0.99 to 1.00.

Table 27. GRADE Summary of Evidence: Test Performance (PPV and NPV) of Conventional Screening for T13

Outcome	Number of Participants and Studies	Effect	Test Accuracy CoE	Rationale
PPV	12,084 participants,[10,14] 2 studies	3.5% and 14.3%	⊕⊕◯◯ **LOW**	Downgraded 1 level each for risk of bias and imprecision (i.e., wide CIs)
NPV	12,084 participants,[10,14] 2 studies	99.99% and 100%	⊕⊕⊕◯ MODERATE	Downgraded 1 level for risk of bias

Abbreviations. CI: confidence interval; CoE: certainty of evidence; NPV: negative predictive value; PPV: positive predictive value.

Table 28. GRADE Summary of Evidence: Test Accuracy for All Common Trisomies (T21, T18, and T13) in Twin Pregnancies

Test Results	Number of Results per 1,000 Patients Tested (95% CI) Prevalence 5.73% Seen in This Study	Number of Participants and Studies	Certainty of Evidence (GRADE)	Rationale
True positives	52 (34 to 57)	192 participants, 1 study[11]	⊕⊕◯◯ **LOW**	Downgraded 1 level each for risk of bias and imprecision (i.e., wide CIs)
False negatives	5 (0 to 23)			
True negatives	943 (924 to 943)	192 participants, 1 study[11]	⊕⊕⊕◯ **MODERATE**	Downgraded 1 level for risk of bias
False positives	0 (0 to 19)			

Abbreviation. CI: confidence interval. Note. Single study sensitivity: 0.91 (95% CI, 0.59 to 1.00); single study specificity: 1.00 (95% CI, 0.98 to 1.00).

Table 29. GRADE Summary of Evidence: Test Performance (PPV and NPV) of cfDNA Tests for All Common Trisomies (T21, T18, and T13) in Twin Pregnancies

Outcome	Number of Participants and Studies	Effect (95% CI)	Test Accuracy CoE	Rationale
PPV	192 participants, 1 study[11]	100% (NA)	⊕⊕⊕◯ MODERATE	Downgraded 1 level for risk of bias
NPV	192 participants, 1 study[11]	99.5% (96.5% to 99.9%)	⊕⊕⊕◯ MODERATE	Downgraded 1 level for risk of bias

Abbreviations. CI: confidence interval; CoE: certainty of evidence; NA: not applicable; NPV: negative predictive value; PPV: positive predictive value.

Table 30. GRADE Summary of Evidence: Test Accuracy of cfDNA Tests for Sex Chromosomal Aneuploidies

Test Results	Number of Results per 1,000 Patients Tested (95% CI) Prevalence 0.42% Seen in This Study	Number of Participants and Studies	Certainty of Evidence (GRADE)	Rationale
True positives	4 (1 to 4)	474 participants, 1 study[16]	⨁◯◯◯ VERY LOW	Downgraded 1 level each for risk of bias, indirectness (i.e., 45,X only), and imprecision (i.e., wide CIs)
False negatives	0 (0 to 3)			
True negatives	996 (988 to 996)	474 participants, 1 study[16]	⨁◯◯◯ VERY LOW	Downgraded 1 level each for risk of bias, indirectness (i.e., 45,X only), and imprecision (i.e., wide CIs)
False positives	0 (0 to 8)			

Abbreviations. 45,X: Turner syndrome; CI: confidence interval. Note. Single study sensitivity: 1.00 (95% CI, 0.16 to 1.00); single study specificity: 1.00 (95% CI, 0.99 to 1.00).

Table 31. GRADE Summary of Evidence: Test Performance (PPV and NPV) of cfDNA Tests for Sex Chromosomal Aneuploidies

Outcome	Number of Participants and Studies	Effect (95% CI)	Test Accuracy CoE	Rationale
PPV	474 participants, 1 study[16]	100% (NA)	⨁⨁◯◯ LOW	Downgraded 1 level each for risk of bias and indirectness (i.e., 45,X only)
NPV	474 participants, 1 study[16]	100% (NA)	⨁⨁◯◯ LOW	Downgraded 1 level each for risk of bias and indirectness (i.e., 45,X only)

Abbreviations. 45,X: Turner syndrome; CI: confidence interval; CoE: certainty of evidence; NA: not applicable; NPV: negative predictive value; PPV: positive predictive value.

Economic Impact and Cost-Effectiveness

Universal cfDNA screening was more effective than conventional screening in the majority of the economic studies we reviewed, but the results differed depending on whether cfDNA represented value for money (low quality evidence, based on 8 economic studies; Table 32). Universal cfDNA also identified more cases of aneuploidy than conventional screening, resulting in fewer affected live births

because women elected to terminate the affected fetuses. Universal cfDNA also reduced the number of invasive tests performed and associated procedure-related losses.

Table 32. GRADE Summary of Evidence: Cost-Effectiveness

Number of Participants (N) Studies (k)	Findings	Certainty of Evidence	Rationale
Outcome: Cost-Effectiveness			
N > 10,000,000 (women in theoretical cohorts) 8 economic studies[18-25]	cfDNA was more effective than conventional screening but may be more costly.	⨁⨁◯◯ LOW	Downgraded 1 level each for risk of bias and imprecision (i.e., not assessable)

Abbreviation. cfDNA: cell-free DNA.

Clinical Practice Guidelines

A search for clinical practice guidelines related to prenatal screening using cfDNA identified 13 eligible guidelines.[1,26-37] We included any guideline that met basic eligibility criteria and discussed the use of cfDNA in prenatal screening for the general obstetric population. We assessed the majority of clinical practice guidelines as having poor methodological quality due to a lack of reporting on how the evidence base was identified and appraised and how the recommendations were made.[1,27-29,31,32,34-37] We assessed the clinical practice guidelines from the Society of Obstetricians and Gynaecologists of Canada (SOGC) Genetics Committee and the Canadian College of Medical Geneticists (CCMG) as having fair methodological quality due to concerns about the recommendation development process.[26] We assessed the clinical practice guidelines from the Human Genetics Society of Australasia (HGSA) and the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) as having good methodological quality due to minor concerns about recommendation development and stakeholder involvement.[30] We also assessed the screening recommendations from the U.K. National Screening Committee as having good methodological quality with only minor concerns about clarity and applicability.[33]

HGSA and RANZCOG agreed that there was sufficient evidence to support the use of cfDNA in women with singleton pregnancies at 10 weeks' gestation or later as:

- A primary screening test for fetal aneuploidy, or
- A secondary screen for women with an increased probability result on a primary screening test who do not wish to undergo invasive diagnostic testing, or
- Screening in any woman with probability below the traditional threshold for offering diagnostic testing (i.e., less than 1 in 300) who are insufficiently reassured by the results and wish to self-fund further screening[30]

For twin pregnancies, HGSA and RANZCOG recommend that cfDNA-based screening be offered with appropriate pre-test counselling regarding the increased test failure rate and the lack of research data compared with singleton gestations.[30] The choice of a first line screening test, either a combined FTS (cFTS) or cfDNA, will depend on local resources, patient demographics, and individual patient characteristics.[30]

All pregnant women in England are offered the combined test for Down syndrome as part of the NHS Fetal Anomaly Screening Programme (FASP).[33] This program includes FTS for T18 and T13.[33] Pregnant women at higher risk (more than a 1 in 150 chance) of having a baby with 1 of these conditions are offered follow-up diagnostic tests.[33] Currently, cfDNA testing has not been incorporated into routine screening programs in the UK.[33] The UK National Screening committee have commissioned research on the use of cfDNA in routine prenatal screening and will review the results before recommending whether the test can be safely introduced as part of FASP.[33] The committee cited the following reasons why cfDNA is not currently routinely available to the general obstetric population:

- The tests had only been used in women at high risk and international research had not been conducted showing its effectiveness in day to day practice within NHS.
- Testing cfDNA is a relatively new method and the UK does not yet have the resources to support a full screening program. Many of the tests currently offered within the UK are sent abroad for processing.
- The test can take about 2 weeks to process, which may cause unnecessary anxiety for parents-to-be, especially when their baby is at very low risk of having a condition. Therefore, it is unclear whether all pregnant women should be offered cfDNA testing or just those identified as high risk using the combined test.
- Inconclusive results or no-call results may cause further anxiety and delay decisions about whether to undergo other forms of diagnostic testing.[33]

In Canada, SOGC and CCMG recommend that all pregnant women, regardless of age, should be offered the option of prenatal screening for the most common fetal aneuploidies through an informed counselling process.[26] Women and providers should discuss the risks, benefits, and alternatives of the various prenatal diagnoses and screening options, including the option of no testing, before any prenatal screening begins.[26] Following this counseling, patients should be offered the following options:

- No aneuploidy screening
- Standard prenatal screening based on locally offered paradigms
- Ultrasound-guided invasive testing when appropriate indications are present
- Maternal plasma cfDNA screening where available, with the understanding that it may not be provincially funded[26]

SOGC and CCMG also provide recommendations on the type of information that should be given to patients who are considering cfDNA screening, including the implications of a failed test or a positive cfDNA test (Table 33).[26]

The rest of the guidelines, assessed as having poor methodological quality, generally recommend that all pregnant women be informed of the range of screening options, including cfDNA for prenatal screening for trisomies 13, 18, and 21 (Table 33).[1,27-29,31,32,34-37] The guidelines also often emphasize the importance of discussing the implications of testing with a health care professional with expertise in genetic testing and counseling (Table 33).[1,27-29,31,32,34-37] The joint ACOG and Society of Maternal and Fetal Medicine (SMFM) guideline, which was rated as having poor methodological quality, states that[1]:

- Aneuploidy screening or diagnostic testing should be discussed and offered to all women early in pregnancy, ideally at the first prenatal visit (based primarily on consensus and expert opinion)

- All women should be offered the option of aneuploidy screening or diagnostic testing for fetal genetic disorders, regardless of maternal age (based primarily on consensus and expert opinion)
- Screening for aneuploidy should be an informed patient choice, with an underlying foundation of shared decision making that fits the patient's clinical circumstances, values, interests, and goals (based primarily on consensus and expert opinion)
- cfDNA screening should not be used as a substitute for diagnostic testing because of its potential for FP and FN test results (based on good and consistent scientific evidence)
- No method of aneuploidy screening is as accurate in twin gestations as it is in singleton pregnancies
- Analyte screening for fetal aneuploidy should be limited to singleton and twin pregnancies because data are generally unavailable for higher-order multifetal gestations
- All women with a positive cfDNA test result should undergo diagnostic testing before taking any irreversible action, such as pregnancy termination (based on good and consistent scientific evidence)
- Women with a positive screening test result for fetal aneuploidy should be offered further detailed counseling and testing (based on good and consistent scientific evidence)
- Women whose cfDNA screening test results are not reported, are indeterminate, or are uninterpretable (a no-call test result) should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing due to the increased risk of aneuploidy (based on good and consistent scientific evidence)
- Parallel or simultaneous testing with multiple screening methodologies for aneuploidy is not cost-effective and should not be performed (based primarily on consensus and expert opinion)

Various guidelines also discuss the role of cfDNA in prenatal screening for SCAs. The American College of Medical Genetics and Genomics recommends informing all pregnant women, as part of pretest counseling, of the availability of expanded screening for sex chromosome aneuploidies.[29] Conversely, the European Society of Human Genetics and the American Society of Human Genetics guidelines state that:

- "Expanding NIPT-based prenatal screening to also report on sex chromosomal abnormalities and microdeletions not only raises ethical concerns related to information and counseling challenges but also risks reversing the important reduction in invasive testing achieved with implementation of NIPT for aneuploidy, and is therefore currently not recommended."[28]

The use of cfDNA tests to screen for SCAs is also not recommended due to a lack of evidence in the joint guidelines adopted by the Austrian Society of Obstetrics and Gynecology, Austrian Society of Ultrasound in Medicine, Austrian Society of Pre- and Perinatal Medicine, Austrian Society of Human Genetics, German Society of Ultrasound in Medicine, Fetal Medicine Foundation Germany, and the Swiss Society of Ultrasound in Medicine.[35] Other guidelines note the potential discovery of fetal and maternal SCAs that may be of minor or no clinical significance and the lower performance of cfDNA screening in detecting SCAs.[1,26,27,30,31] None of these guidelines make formal recommendations on the use of cfDNA for SCA screening.[1,26,27,30,31]

The organization Choosing Wisely includes 1 recommendation from the SMFM on the use of serum aneuploidy screening after cfDNA screening,[93] which states that serum aneuploidy screening should not

be performed after cfDNA aneuploidy screening.[93] The rationale is that when low-risk results have been reported on either test, there is limited clinical value of performing the other screen. While serum screening may identify some aneuploidies not detected by cfDNA screening, the yield is too low to justify the additional serum screen.[93]

Table 33. Clinical Practice Recommendations on cfDNA Prenatal Screening

Organization	Topic	Excerpted Recommendation(s)	Status
Good Methodological Quality			
Human Genetics Society of Australia, Royal Australian and New Zealand College of Obstetricians and Gynaecologists[30]	Prenatal screening and diagnostic testing for fetal chromosomal and genetic conditions	• Acceptable first-line screening tests for fetal chromosome abnormalities in the first trimester include either: a) combined FTS with nuchal translucency and serum PAPP-A and β-hCG measurements, or b) cfDNA-based screening • The choice of first-line screening test will depend on local resources, patient demographics, and individual patient characteristics. • Pre-test counselling for cfDNA-based screening should include informed decision making regarding testing for fetal sex and sex chromosome aneuploidy. The potential for other unanticipated findings of relevance to maternal health (including maternal genomic imbalances), should be included in pre-test counselling. • Acceptable first-line screening tests for chromosome conditions in second trimester include: a) maternal serum screening (MA + AFP + βhCG +UE3 +/- Inhibin) and, b) cfDNA-based screening. • The choice of first-line screening test will depend on local resources, patient demographics, and individual patient characteristics. • The option of cfDNA-based screening as a second-tier test should be discussed with all women at increased probability of a chromosome condition after primary screening. The advantages and disadvantages of second tier cfDNA-based screening, compared with diagnostic testing, or no further assessment, should be discussed by a clinician with appropriate expertise. • Diagnostic testing with amniocentesis or chorionic villus sampling should be recommended prior to definitive management decisions (e.g. termination of pregnancy) in cases of "increased chance" screening results, including cfDNA-based screening. • In twin pregnancies, cfDNA-based screening may be offered with appropriate pre-test counselling regarding an increased test failure rate, and less available performance data compared with singletons.	Adopted in 2018, due for updating in 2021 or as required
NHS Fetal Anomaly Screening Programme[33]	cfDNA testing for Down syndrome and other trisomies	• Although cfDNA is thought to be very accurate, there is still a chance that it would incorrectly identify a pregnancy as high risk of Down's syndrome. For this reason it should not replace the current diagnostic test used in FASP. Its improved accuracy compared to the combined test does mean that fewer women will go on to have	Guidance issued in 2015 with updates published in 2019

Organization	Topic	Excerpted Recommendation(s)	Status
		the invasive diagnostic test when their baby does not in fact have Down's syndrome. • There is the potential for cfDNA to replace the current combined screening test in the future. However, as the technology stands, the number of tests which don't give a result would mean that more women would be offered invasive testing than now. • Also, cfDNA may be very accurate when identifying which babies are at a higher risk of Down's syndrome, but there is not enough evidence to be sure of its accuracy when looking for Edwards' syndrome and Patau's syndrome. • The UK National Screening Committee will continue to keep emerging evidence under review.	
Fair Methodological Quality			
Society of Obstetricians and Gynaecologists of Canada, Canadian College of Medical Geneticists[26]	Prenatal screening for fetal aneuploidy, fetal anomalies, and adverse pregnancy outcomes	• All pregnant women in Canada, regardless of age, should be offered, through an informed counselling process, the option of a prenatal screening test for the most common fetal aneuploidies. • First-trimester nuchal translucency should be interpreted for risk assessment only when measured by sonographers or sonologists trained and accredited for this fetal screening service and when there is ongoing quality assurance. For aneuploidy, it should be offered as a screen with maternal serum biochemical markers in singleton pregnancies. • Maternal age alone is a poor minimum standard for prenatal screening for aneuploidy, and it should not be used as a basis for recommending invasive fetal diagnostic testing when prenatal screening for aneuploidy is available. • Health care providers should be aware of the prenatal screening modalities available in their province or territory. A reliable prenatal system needs to be in place ensuring timely reporting of results. Prenatal screening programs should be implemented with resources that support audited screening and diagnostic laboratory services, ultrasound, genetic counselling services, patient and health care provider education, and high-quality diagnostic testing, as well as resources for administration, annual clinical audit, and data management. In addition, there must be the flexibility and funding opportunities to adjust the program to new technology and protocols. • A discussion of the risks, benefits, and alternatives of the various prenatal diagnoses and screening options, including the option of no testing, should be undertaken with all patients prior to any prenatal screening. Following this counselling, patients should be offered (1) no aneuploidy screening, (2) standard prenatal screening based on locally-offered paradigms, (3) ultrasound-guided	Adopted in 2017 with a review date of 2022

Organization	Topic	Excerpted Recommendation(s)	Status

		invasive testing when appropriate indications are present, or (4) maternal plasma cfDNA screening where available, with the understanding that it may not be provincially funded.
		• Regardless of aneuploidy screening choice, all women should be offered a fetal ultrasound (optimally between 11 and 14 weeks) to confirm viability, gestational age, number of fetuses, chorionicity in multiples, early anatomic assessment, nuchal translucency evaluation where available. The nuchal translucency measurement for aneuploidy risk estimation (combined with maternal serum) should not be performed if cfDNA screening has been used. Every effort should be made to improve access to high-quality first trimester ultrasound for all Canadian women. In areas where nuchal translucency assessment is not available, a first trimester dating ultrasound improves the accuracy of maternal serum screening and the management of pregnancy.
		• A large nuchal translucency (>3.5 mm) should be considered a major marker for fetal chromosomal and structural anomalies and requires genetic counselling, an offer of invasive testing with chromosomal microarray analysis, and detailed second-trimester ultrasound follow-up.
		• Women who are considering undergoing maternal plasma cfDNA screening should be informed that:
		  • It is a highly effective screening test for the common fetal trisomies (21, 18, 13), performed after 10 weeks' gestation.
		  • There is a possibility of a failed test (no result available), FN or FP fetal result, and an unexpected fetal or maternal result.
		  • All positive cfDNA screening results should be confirmed with invasive fetal diagnostic testing prior to any irrevocable decision.
		  • Management decisions, including termination of pregnancy, require diagnostic testing and should not be based on maternal plasma cfDNA results alone because it is not a diagnostic test.
		  • If a fetal structural abnormality is identified in a woman regardless of previous screening test results, the woman should undergo genetic counselling and be offered invasive diagnostic testing with rapid aneuploidy detection and reflex to microarray analysis if rapid aneuploidy detection is normal or inconclusive.
		  • Although cfDNA screening for aneuploidy in twin pregnancy is available, there is less validation data than for a singleton pregnancy and it should be undertaken with caution.
		  • Routine cfDNA screening for fetal microdeletions is not currently recommended.	

Organization	Topic	Excerpted Recommendation(s)	Status
		• If a fetal structural abnormality is identified, regardless of previous screening test results, genetic counselling and invasive fetal diagnostic testing should be offered with rapid aneuploidy detection, and chromosomal microarray analysis should be considered to confirm those malformations associated with a high frequency of abnormal results. • The sonographic "soft markers" of echogenic intracardiac focus and chorionic plexus cysts should not be used to adjust the a priori risk for fetal aneuploidy. • Universal screening for adverse pregnancy outcomes using maternal serum markers is currently not recommended outside of an investigational protocol with informed consent.	
Poor Methodological Quality			
American College of Medical Genetics and Genomics (ACMG)[29]	Noninvasive prenatal screening for fetal aneuploidy	ACMG recommends: • Allowing patients to select diagnostic or screening approaches for the detection of fetal aneuploidy and/or genomic changes that are consistent with their personal goals and preferences. • Informing all pregnant women that NIPS is the most sensitive screening option for traditionally screened aneuploidies (i.e., Patau, Edwards, and Down syndromes). • Referring patients to a trained genetics professional when an increased risk of aneuploidy is reported after NIPS. • Offering diagnostic testing when a positive screening test result is reported after NIPS. • Providing accurate, balanced, up-to-date information, at an appropriate literacy level when a fetus is diagnosed with a chromosomal or genomic variation in an effort to educate prospective parents about the condition of concern. These materials should reflect the medical and psychosocial implications of the diagnosis. • Informing all pregnant women, as part of pretest counseling for NIPS, of the availability of the expanded use of screening for sex chromosome aneuploidies. • Providers should make efforts to deter patients from selecting sex chromosome aneuploidy screening for the sole purpose of biologic sex identification in the absence of a clinical indication for this information. • Informing patients about the causes and increased possibilities of false-positive results for sex chromosome aneuploidies as part of pretest counseling and screening for these conditions. Patients should also be informed of the potential for results of conditions that, once confirmed, may have a variable prognosis (e.g., Turner syndrome) before consenting to screening for sex chromosome aneuploidies.	Adopted in 2016 with no specific review date listed

Organization	Topic	Excerpted Recommendation(s)	Status
		• Referring patients to a trained genetics professional when an increased risk of sex chromosome aneuploidy is reported after NIPS. ACMG does not recommend: • NIPS to screen for autosomal aneuploidies other than those involving chromosomes 13, 18, and 21.	
American College of Obstetricians and Gynecologists, Society for Maternal–Fetal Medicine[1]	Screening for fetal aneuploidy	• Screening for aneuploidy should be an informed patient choice, with an underlying foundation of shared decision making that fits the patient's clinical circumstances, values, interests, and goals. • Aneuploidy screening or diagnostic testing should be discussed and offered to all women early in pregnancy, ideally at the first prenatal visit. • All women should be offered the option of aneuploidy screening or diagnostic testing for fetal genetic disorders, regardless of maternal age. • Some women who receive a positive test result from traditional screening may prefer to have cfDNA screening rather than undergo definitive testing. This approach may delay definitive diagnosis and management and may fail to identify some fetuses with aneuploidy. • Parallel or simultaneous testing with multiple screening methodologies for aneuploidy is not cost-effective and should not be performed. • Women who have a negative screening test result should not be offered additional screening tests for aneuploidy because this will increase their potential for a FP test result. • Women who undergo FTS should be offered second-trimester assessment for open fetal defects (by ultrasonography, maternal serum alpha-fetoprotein screening, or both) and ultrasound screening for other fetal structural defects. • Because cfDNA is a screening test, it has the potential for FP and FN test results and should not be used as a substitute for diagnostic testing. • All women with a positive cfDNA test result should have a diagnostic procedure before any irreversible action, such as pregnancy termination, is taken. • Women whose cfDNA screening test results are not reported, are indeterminate, or are uninterpretable (a no call test result) should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy. • Women with a positive screening test result for fetal aneuploidy should be offered further detailed counseling and testing. • No method of aneuploidy screening is as accurate in twin gestations as it is in singleton pregnancies. Because data generally are unavailable for higher-order	Adopted in 2016 with no specific review date listed

Organization	Topic	Excerpted Recommendation(s)	Status
		multifetal gestations, analyte screening for fetal aneuploidy should be limited to singleton and twin pregnancies.	
Society for Maternal-Fetal Medicine[37]	Role of ultrasound in women who undergo cfDNA screening	• In women who have already received a negative cfDNA screening result, ultrasound at 11 to 14 weeks of gestation solely for the purpose of nuchal translucency measurement (CPT code 76813) is not recommended. • Diagnostic testing should not be recommended to patients solely for the indication of an isolated soft marker in the setting of a negative cfDNA screen. • In women with an isolated soft marker that has no other clinical implications (i.e., choroid plexus cyst or echogenic intracardiac focus) and a negative cfDNA screen, we recommend describing the finding as not clinically significant or as a normal variant. • In women with an isolated soft marker without other clinical implications (i.e., choroid plexus cyst or echogenic intracardiac focus) and a negative first- or second-trimester screening result, we recommend describing the finding as not clinically significant or as a normal variant. • We recommend that all women in whom a structural abnormality is identified by ultrasound be offered diagnostic testing with chromosomal microarray.	Adopted in 2017 with no specific review date listed
Austrian Society of Obstetrics and Gynecology, Austrian Society of Ultrasound in Medicine, Austrian Society of Pre- and Perinatal Medicine, Austrian Society of Human Genetics, German Society of Ultrasound in Medicine, Fetal Medicine Foundation Germany, Swiss Society of Ultrasound in Medicine[35]	Cell-Free DNA testing for fetal chromosomal anomalies	• cfDNA testing should be offered only after, or in conjunction with, a qualified ultrasound and following appropriate counseling about the nature, scope and significance of the test. • cfDNA tests are screening tests. A high-risk cfDNA testing result should always be confirmed by an invasive diagnostic test (Chorionic villous sampling, amniocentesis), before a clinical consequence is drawn from the findings. • cfDNA testing can be used as secondary screening test for trisomy 21 (Down syndrome) for the reduction of invasive procedures after a high or intermediate risk result from First-trimester combined test (1 in 1,000 or > 1:500). It should be noted that, even when cfDNA testing is used as a secondary screening, invasive diagnostic testing (Chorionic villous sampling, amniocentesis) is still the method of choice when the adjusted risk for T21 after the combined test is > 1:10 or the fetal nuchal translucency thickness is > 3.5 mm or a fetal malformation is present. • cfDNA tests can also be used as a primary screening method for fetal T21 in pregnant women of every age and risk group. • In general, it should be noted that the performance of cfDNA screening for trisomy 18 (Edwards syndrome) and T13 (Patau syndrome) is lower than that for T21. • Based on the available evidence the use of cfDNA tests to screen for aneuploidy of sex chromosomes and microdeletion syndromes can currently not be recommended without reservation.	Adopted in 2015 with no specific review date listed

Organization	Topic	Excerpted Recommendation(s)	Status
Chromosome Abnormality Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis[27]	Screening tests for detecting fetal chromosome abnormalities	The following protocol options are currently considered appropriate: • cfDNA screening as a primary test offered to all pregnant women, completed weeks (e.g. 10 = 10 weeks 0 days to 10 weeks 6 days) • cfDNA secondary to a high-risk assessment based on serum and ultrasound screening protocols • cfDNA contingently offered to a broader group of women ascertained as having high or intermediate risks by conventional screening; contingent provision of cfDNA could also include a protocol in which women with very high risks are offered invasive prenatal diagnosis, while those with intermediate risk are offered cfDNA	Adopted in 2015 with no specific review date listed
European Society of Human Genetics, American Society of Human Genetics[28]	Noninvasive prenatal testing for aneuploidy	• 1. NIPT offers improved accuracy when testing for common autosomal aneuploidies compared with existing tests such as cFTS. However, a positive NIPT result should not be regarded as a final diagnosis: FPs occur for a variety of reasons (including that the DNA sequenced is both maternal and fetal in origin, and that the fetal fraction derives from the placenta as well as the developing fetus). Thus women should be advised to have a positive result confirmed through diagnostic testing, preferably by amniocentesis, if they are considering a possible termination of pregnancy. • 2. The better test performance, including lower invasive testing rate of NIPT-based screening should not lead to lower standards for pretest information and counseling. This is especially important in the light of the aim of providing pregnant women with meaningful options for reproductive choice. There should be specific attention paid to the information needs of women from other linguistic and cultural backgrounds or who are less health literate. • 3. If NIPT is offered for a specific set of conditions (e.g., trisomies 21, 18 and 13), it may not be reasonably possible to avoid additional findings, such as other chromosomal anomalies or large scale insertions or deletions. As part of pretest information, women and couples should be made aware of the possibility of such additional findings and the range of their implications. There should be a clear policy for dealing with such findings, as much as possible also taking account of pregnant women's wishes with regard to receiving or not receiving specific information. • 4. Expanding NIPT-based prenatal screening to also report on SCAs and microdeletions not only raises ethical concerns related to information and counseling challenges but also risks reversing the important reduction in invasive testing achieved with implementation of NIPT for aneuploidy, and is therefore currently not recommended.	Adopted in 2014 with no specific review date listed

Organization	Topic	Excerpted Recommendation(s)	Status
		• 5. Emerging opportunities for combining prenatal screening for fetal abnormalities with screening aimed at prevention may undermine adequate counseling by sending mixed messages. The objective of any prenatal screening activity should be made explicit and, as far as possible, forms of prenatal screening with different aims should be presented separately. If not physically possible, this separation should at least be made conceptually when providing the relevant information. • 6. In countries where prenatal screening for fetal abnormalities is offered as a public health programme, governments and public health authorities should adopt an active role to ensure the responsible introduction of NIPT as a second or first-tier screening test for Down syndrome and other common autosomal aneuploidies. This entails ensuring quality control also extending to the non-laboratory aspects of NIPT-based prenatal screening (information, counseling), education of professionals, systematic evaluation of all aspects of the screening programme, as well as promoting equity of access for all pregnant women within the confines of the available budget, and setting up a governance structure for responsible further innovation in prenatal screening. • 7. Different scenarios for NIPT-based screening for common autosomal aneuploidies are possible, including NIPT as an alternative first-tier option. The inevitable trade-offs underlying those scenarios should not just be regarded as a matter of screening technology and health economics; the question is also how these trade-offs enable or impede meaningful reproductive choices and how they affect both the balance of benefits and burdens for pregnant women and their partners, and the screening goals and values acceptable to society. • 8. In order to adequately evaluate prenatal screening practices, there is a need to further develop and validate measures of informed choice as well as interventions aimed at enabling informed choices. The transition to NIPT-based prenatal screening presents an opportunity to fill this gap in knowledge. • 9. In the light of sequencing technologies becoming better and cheaper, there is an acute need for a proactive professional and societal debate about what the future scope of prenatal screening for fetal abnormalities should be. As argued […], there are strong ethical reasons for not expanding the scope of prenatal screening beyond serious congenital and childhood disorders. • 10. The scenario in which prenatal screening would open up possibilities for fetal therapy in addition to autonomous reproductive choice raises fundamental questions about the relation between reproductive autonomy and parental responsibility that require an in depth proactive ethical analysis.	

Organization	Topic	Excerpted Recommendation(s)	Status
International Society of Ultrasound in Obstetrics and Gynecology[34]	cfDNA aneuploidy testing: impact on screening policies and prenatal ultrasound	All women should be offered a first-trimester ultrasound scan according to ISUOG guidelines, regardless of their intention to undergo cfDNA testing. • If the woman has had a negative cfDNA test result, nuchal translucency thickness should still be measured and reported as a raw value and centile. The management of increased nuchal translucency with a normal cfDNA test result is currently based on local guidelines. However, it is not necessary to compute first-trimester risk estimates for trisomies 21, 18 and 13 based on nuchal translucency measurements and maternal biochemistry in a woman known to have a normal cfDNA result. Accordingly, soft markers for T21 should not be assessed in women with a normal cfDNA test result due to their high FP rate and poor positive predictive value. • If the woman has not had a cfDNA test, pretest counseling is essential. Various options regarding screening or testing for T21 and, to a lesser extent, trisomies 18 and 13 should be explained clearly, including information on the expected test performance, potential adverse effects, and pros and cons of each option. Following a normal first-trimester scan, as defined by ISUOG guidelines, three options might be considered for women who wish to have further risk assessment: (1) Screening strategies based on individual risk calculated from maternal age and nuchal translucency measurement and/or maternal serum markers and/or other ultrasound markers in the first trimester (defined by the conventional crown–rump length range of 45–84 mm). Following such screening, women can be offered a choice, according to their calculated individual risk, of having no further testing, cfDNA testing or invasive testing. Cut-offs, defining two (low/high risk) or three (low/intermediate/high risk) groups, should be defined on a local/national basis and will be affected by public health priorities and available resources. Offering cfDNA testing should always be balanced with the potential and risk of conventional karyotyping, with or without microarray analysis, following invasive sampling. More importantly, the role of cfDNA testing as an alternative to standard invasive testing in women considered to be at very high risk after combined screening (>1:10) but with no ultrasound anomaly should be evaluated in prospective studies. Expert opinion currently suggests that cfDNA testing should not replace routinely invasive testing in this group, based on the fact that, in this population, only 70% of the chromosomal abnormalities are trisomy 21, 18 or 13, and that chromosomal microarray analysis, if offered, is able to detect a large number of additional anomalies. (2) cfDNA testing as a first-line screening test. Most current guidelines endorse cfDNA testing only for high- or intermediate-risk populations, for which comprehensive data exist. Experience in low-risk	Adopted in 2017, updates produced on a regular basis but no specific review date listed

Organization	Topic	Excerpted Recommendation(s)	Status
		populations is increasing, apparently confirming the high detection rates published for high-risk populations. However, testing in low-risk women may impact on the quality of both pretest counseling and subsequent ultrasound screening. In particular, cfDNA testing should not replace first-trimester ultrasound and should not be offered when an ultrasound anomaly or markedly increased nuchal translucency is detected. Using cfDNA in low-risk patients might be endorsed as a widely available option only when more data emerge and cfDNA costs decrease. (3) Invasive testing based on a woman's preference or background risk (maternal age, previous history, fetal ultrasound anomaly) with no further individual risk calculation. An invasive test might be discussed in light of the recently reported reduction in the risk of invasive procedures, as well as the increase in cytogenetic resolution provided by microarray techniques. However, the cost of this option is not usually covered by most national insurance policies and it should not be recommended beyond the context of clinical trials and until sufficient peer-reviewed data and validation studies have been published. • cfDNA test results should always be interpreted and explained individually in relation to the a-priori risk and the fetal fraction. • In the presence of a fetal structural anomaly, the indications for fetal karyotyping and/or microarray testing should not be modified by a previously normal cfDNA test result. • In the case of a failed cfDNA test, the patient should be informed about the increased risk of anomalies as well as alternative screening and testing strategies. • cfDNA testing is not diagnostic, and confirmatory invasive testing is required in the presence of an abnormal result. Whenever there is discordance between an abnormal cfDNA test result and a normal ultrasound examination, amniocentesis rather than chorionic villus sampling should be performed. • Accuracy of cfDNA testing in twin pregnancies should be investigated further. • Variations in cfDNA test performance by different providers should be investigated further. It is becoming technically feasible to test non-invasively, not only for trisomies but also for other genetic syndromes. Both healthcare providers and women should be clearly aware of the tests being performed and of their performance, as having multiple tests increases the overall FP rate and failure rate. The detection rate for microdeletions has yet to be established and most national guidelines currently do not support testing for microdeletions on cfDNA. Screening for microdeletions also raises complex issues regarding pretest and post-test counseling.	

Organization	Topic	Excerpted Recommendation(s)	Status
		• Prospective, publicly funded studies assessing the cost-effectiveness of various screening strategies should be performed as a matter of urgency.	

Israeli Society of Medical Genetics NIPT Committee[31]	Non-invasive prenatal testing of cfDNA in maternal plasma for detection of fetal aneuploidy	• NIPT should be considered for women at high risk for fetal chromosomal abnormalities, in singleton pregnancies, from 10 weeks of gestation. The following categories are considered high risk:
		  • Maternal age of 35 years or above at the time of conception.
		  • Sonographic 'soft markers' of chromosomal anomaly (such as intracardiac echogenic foci, mild pyelectasis, etc.).
		  • Personal or familial history of a chromosomal anomaly detectable by NIPT.
		  • Abnormal Down syndrome screening result (first or second trimester).
		  • A parent carrier of a Robertsonian translocation involving chromosomes 13 or 21
			Adopted in 2014 with no specific review date listed

National Society of Genetic Counselors[32]	Prenatal cfDNA screening	• The National Society of Genetic Counselors supports prenatal cfDNA screening, also known as NIPT or NIPS, as an option for pregnant patients. • Because cfDNA screening cannot definitively diagnose or rule out genetic conditions, qualified providers should communicate the benefits and limitations of cfDNA screening to patients prior to testing. • Many factors influence cfDNA screening performance, therefore it may not be the most appropriate option for every pregnancy. • Prior to undergoing cfDNA screening, patients should have the opportunity to meet with qualified prenatal care providers who can facilitate an individualized discussion of patients' values and needs, including the option to decline all screening or proceed directly to diagnostic testing. • Clinicians with expertise in prenatal screening, such as genetic counselors, should provide post-test genetic counseling to patients with increased-risk screening results. • Diagnostic testing should be offered to patients with increased-risk results to facilitate informed decision making.	Adopted in 2016 with no specific review date listed
Polish Gynecological Society, Polish Human Genetics Society[36]	cfDNA testing in prenatal diagnosis	• NIPT should not replace FTS based on fetal ultrasound scan and biochemical testing of maternal blood. NIPT should be ordered by a physician who has experience in obstetrics, perinatology or clinical genetics. • NIPT should be performed between the 10th and 15th week of pregnancy. NIPT is not recommended for low risk pregnancies with a risk less than 1:1000 as indicated by integrated tests (ultrasound+ biochemical testing of maternal blood). • NIPT should be offered to pregnant women with a risk of fetal chromosomal aberration from 1:100 to 1:1000. If the risk is higher than 1: 100, invasive prenatal diagnosis should be offered. When fetal congenital anomalies are diagnosed based	Adopted in 2017 with no specific review date listed

Organization	Topic	Excerpted Recommendation(s)	Status
		on ultrasound but the NIPT results are correct, the patient must be referred to a genetics specialist for further diagnostics and genetic counselling. • NIPT is not recommended for multiple pregnancies (triplets and higher). • Before NIPT ultrasound scan should be performed to assess the number of fetuses and the gestational age. • NIPT should not replace fetal ultrasound examination. Ultrasound scan has to be performed following the guidelines of the Ultrasound Section of the Polish Gynaecological Society. • When NIPT results could not be obtained (up to 5%) the NIPT test may be repeated or invasive diagnostics has to be offered. • NIPT and invasive diagnostics should not be performed at the same time. When NIPT shows high risk of chromosomal aberration amniocentesis is indicated as a method of invasive diagnostics. • When NIPT estimates high risk of fetal chromosomal aberration the patient has to be consulted by clinical geneticist or specialist in perinatology. • Pregnancy cannot be terminated based only on NIPT result. NIPT results should be signed by a specialist in medical laboratory diagnostics.	

Abbreviations. 45,X: Turner syndrome; ACMG: American College of Medical Genetics and Genomics; AFP: alpha-fetoprotein; fDNA: cell-free DNA; cFTS: combined first-trimester screen; CPT: Current Procedural Terminology; FASP: Fetal Anomaly Screening Programme; FN: false negative; FP: false positive; FTS: first-trimester screen; hCG: human chorionic gonadotropin; ISUOG: International Society of Ultrasound in Obstetrics and Gynecology; MA: maternal age; NIPS: noninvasive prenatal screening; NIPT: noninvasive prenatal testing; PAPP-A: pregnancy-associated plasma protein A; SCA: sex chromosome abnormality; T13: trisomy 13; T18: trisomy 18; T21: trisomy 21; UE3: estriol.

Selected Payer Coverage Determinations

We did not identify any Medicare National or Local Coverage Determinations related to prenatal screening with cfDNA. Of the 3 private payers that we reviewed, we found detailed policies on prenatal screening using cfDNA from Aetna and Cigna, but only limited detail from Regence.[38-40]

Aetna considers the use of cfDNA screening (e.g., MaterniT21, MaterniT21 PLUS, verifi Prenatal Test, Harmony Prenatal Test, Panorama Prenatal Test, QNatal Advanced) medically necessary for testing for T21, T18, and T13 in pregnant women with single gestations who meet any of the following indications:

- Fetal ultrasonographic findings predicting an increased risk of fetal aneuploidy (i.e., absent or hypoplastic nasal bone, choroid plexus cyst, echogenic bowel, echogenic intracardiac focus, fetal pyelectasis, nuchal translucency, nuchal fold, ventriculomegaly, and shortened femur or humerus), or
- History of a prior pregnancy with an aneuploidy, or
- Parental balanced Robertsonian translocation with increased risk for fetal T13 or T21, or
- Positive screening test for an aneuploidy, including first trimester, sequential, or integrated screen, or a positive quadruple screen, or
- Pregnant women age 35 years and older at expected time of delivery[38]

Aetna considers cfDNA testing to be experimental and investigational for other conditions and indications not listed above (e.g., low-risk women, women with multiple gestations) because its effectiveness has not been established in these circumstances.[38]

Cigna considers cfDNA screening tests for T21, T18, and T13 (e.g., verifi, MaterniT21 Plus, Harmony, Panorama, InformaSeqsm, VisibiliT) to be medically necessary in viable, single gestation pregnancies ≥ 10 weeks' gestation.[39] Screening tests using cfDNA for T21, T18, and T13 at an in-network benefit level when performed in an out-of-network laboratory is considered not medically necessary when the tests are available in an in-network laboratory.[39] Cigna considers cfDNA screening tests for any other indication, including but not limited to the following, to be experimental, investigational, or unproven:

- Multiple gestation
- Screening for a sex-chromosome aneuploidy
- Vanishing twin syndrome
- Screening for T7, T9, T16, or T22
- Screening for microdeletions
- Single-gene disorders
- Whole genome NIPT
- When used to determine the genetic cause of miscarriage (e.g., missed abortion, incomplete abortion)[39]

Cigna also requires that genetic counseling be recommended to individuals considering genetic screening for fetal aneuploidy.[39]

Regence considers the use of cfDNA screening for fetal sex chromosome aneuploidies (e.g. sex chromosome aneuploidy or sex chromosome aneuploidy panel [SCAP] testing) to be investigational.[40] We were not able to identify a publicly available coverage policy from Regence on cfDNA screening for

T21, T18, or T13, but they confirmed that testing for fetal trisomy aneuploidy screening, without criteria or review, is universally covered (Regence staff, personal communication).

Ongoing Trials

We searched the ClinicalTrials.gov database for ongoing studies related to prenatal screening using cfDNA in general obstetric populations (Appendix F). We identified 1 ongoing RCT eligible for this evidence review, but it is not expected to be published until after December 2021 (Table 34).

Table 34. Included Ongoing Studies of Screening Using cfDNA

NCT Number Study Name Study Type	Participants	Treatment Groups	Outcomes	Enrollment	Study Completion Date
NCT03831256[41] PEGASUS-2 RCT	Pregnant women with singleton pregnancies opting for prenatal screening	First tier cfDNA screening (test not specified) Second tier cfDNA screening (test not specified) after a positive conventional FTS	• Gestational age at diagnosis • No-call tests • Length of time between a FP screening result and confirmation of diagnosis • Quality of life • Patient experience • Rate of invasive testing	10,000	December 2021

Abbreviations. FP: false positive; FTS: first-trimester screening; RCT: randomized controlled trial.

Conclusions

Universal cfDNA screening identifies fetuses with any of the trisomies 21, 18, and 13 with lower FP results (low-quality evidence) and more accurately than conventional FTS. Universal cfDNA screening also reduces the rate of subsequent invasive testing (low-quality evidence) due to its lower rates of FPs at the screening stage compared with conventional screening. Universal cfDNA screening had a higher PPV than conventional screening for trisomies 21 18, and 13 (very-low- to moderate-quality evidence). Although the PPV of a screening test will be lower for low prevalence conditions, the PPV of cfDNA screening for trisomies 21, 18, and 13 or sex chromosome aneuploidies was consistently 75% or higher, which was much higher than the PPVs of conventional screening at 3.5% to 28.3% (very-low- to moderate-quality evidence).

We did not find evidence of direct harms from the use of cfDNA tests to identify chromosomal aneuploidies. Similarly, we found a paucity of evidence regarding variations in the effectiveness and harms of cfDNA screening among relevant subpopulations. We found similar results for screening effectiveness and subsequent testing in the models used to determine cost-effectiveness. The economic studies depended on whether cfDNA screening for trisomies 21, 18, and 13 represented value for money (low-quality evidence). Evidence was lacking regarding the cost-effectiveness of universal cfDNA screening for sex chromosome aneuploidies.

In 2018, the average costs paid per cfDNA test in Washington State were $482 for Medicaid populations and $553 for the Public Employees Benefit Board Uniform Medical Plan. The costs were not dissimilar to the $549 threshold at which cfDNA becomes less costly than standard screening, as determined in an economic study at low risk of bias. The study took a societal perspective and included the direct medical costs of screening, diagnosis, and termination of T21 pregnancies as well as the lifetime costs associated with T21.[24]

Good-methodological-quality guidelines differed in their recommendations on the use of cfDNA as a primary screening tool, with 1 guideline recommending its use and 1 guideline deferring universal use until the impact of the method's adoption has been explored. The 1 fair-methodological quality guideline recommends the use of primary cfDNA screening where available but recognizes that it may not be funded by the healthcare system. None of the guidelines recommended the use of cfDNA screening for SCAs, although women could be made aware of the option. The policies from private payers on the use of cfDNA as a universal screening tool for trisomies 21, 18, and 13 also varied, with Aetna restricting the test's use to women known to be at high risk and Cigna covering the use of cfDNA for all pregnant women. Both coverage policies consider the use of cfDNA to be experimental and investigational for multifetal pregnancies. All 3 private payers consider the use of cfDNA to be experimental and investigational for SCAs.

We found 1 ongoing RCT evaluating universal cfDNA in women not known to be at high risk of aneuploidies.

Based on the evidence reviewed in this report, universal screening with cfDNA appears to be an accurate method of screening for the common trisomies T21, T18, and T13 in general obstetric populations. However, universal cfDNA testing is likely to be more expensive than conventional screening, depending on the exact costs of the cfDNA test used. Policy makers therefore need to consider the value of expanding cfDNA screening to all pregnant women and whether it is worth the additional associated costs. The economics studies in this report suggest that universal cfDNA screening can be cost-effective, particularly when the lifetime costs of trisomies T21, T18, and T18 and the wider societal costs are included. However, there is a lack of clinical and cost-effectiveness evidence on the use of cfDNA screening for sex chromosome aneuploidies.

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Appendix A. Search Strategy

Databases

- Ovid MEDLINE, Ovid MEDLINE In-Process & Other Non-Indexed Citations, and Ovid MEDLINE Epub Ahead of Print
- Cochrane Database of Systematic Reviews
- Cochrane Central Register of Controlled Trials
- Scopus
- NHS Economic Evaluation Database and the NIHR Health Technology Assessment Program

Search Terms for Ovid MEDLINE

1. Cell-Free Nucleic Acids/

2. (cell-free dna or cell free dna).ti,ab,kw,kf.

3. (cfdna or cf-dna).ti,ab,kw,kf.

4. (cell-free f?etal dna or cell free f?etal dna).ti,ab,kw,kf.

5. (cffdna or cff-dna).ti,ab,kw,kf.

6. (cirdna or cir-dna).ti,ab,kw,kf.

7. (Cell Free Nucleic Acid? or Cell-free nucleic acid?).ti,ab,kw,kf.

8. circulating nucleic acid?.ti,ab,kw,kf.

9. circulating dna.ti,ab,kw,kf.

10. (ffdna or ff-dna).ti,ab,kw,kf.

11. f?etal free dna.ti,ab,kw,kf.

12. f?etal-free dna.ti,ab,kw,kf.

13. ((non?invasive or non-invasive) adj2 pre?natal).ti,ab,kw,kf.

14. Maternal Serum Screening Tests/

15. nipd.ti,ab,kw,kf.

16. nipt.ti,ab,kw,kf.

17. ((non?invasive or non-invasive) adj2 ante?natal).ti,ab,kw,kf.

18. ((non?invasive or non-invasive) adj2 (f?etal or f?etus)).ti,ab,kw,kf.

19. (Maternal adj1 (blood or plasm*) adj2 (Screen* or test* or sequenc*)).ti,ab,kw,kf.

20. (Bambini* or ClariTest* or Harmony* Prenatal Test or Prenatal Harmony test or informaSeq* or IONA* Test or MaterniT21* or NIFTY* or Panorama* or PrenaTest* or Prequel Prenatal Screen or QNatal* Advanced or Veracity or verifi Prenatal Test or verifi Plus Prenatal Test or VisibiliT*).ti,ab,kw,kf.

21. or/1-20

22. genetic testing/

23. ((genetic* or gene*1 or genome*1 or genomic*) adj2 (test or tests or testing or diagnos* or screen*)).ti,ab,kw,kf.

24. prenatal diagnosis/

25. ((ante?natal or pre?natal or intra?uterine) adj2 (test or tests or testing or diagnos* or detect* or screen*)).ti,ab,kw,kf.

26. or/22-25

27. (noninvasive* or non-invasive*).ti,ab,kw,kf.

28. 26 and 27

29. or/21,28

30. exp aneuploidy/

31. aneuploid*.ti,ab,kw,kf.

32. (trisom* or chromosom* triplicat*).ti,ab,kw,kf.

33. ("47,XY,+21" or " 47,XX,+21").ti,ab,kw,kf.

34. down* syndrome*.ti,ab,kw,kf.

35. Down Syndrome/bl, di [Blood, Diagnosis]

36. exp Sex Chromosome Disorders/

37. chromosome aberrations/

38. klinefelter* syndrome*.ti,ab,kw,kf.

39. XXy syndrome*.ti,ab,kw,kf.

40. XXyy syndrome*.ti,ab,kw,kf.

41. (("48,XXYY" or "49,XXXXY") adj1 syndrome*).ti,ab,kw,kf.

42. XXXY Male*.ti,ab,kw,kf.

43. Turner* Syndrome*.ti,ab,kw,kf.

44. ((Ullrich-Turner or Ullrich Turner) adj1 syndrome).ti,ab,kw,kf.

45. "Gonadal Dysgenesis, 45,X".ti,ab,kw,kf.

46. ("Gonadal Dysgenesis, XO" or XO Gonadal Dysgenesis).ti,ab,kw,kf.

47. "45,x gonadal dysgenesis".ti,ab.

48. "45,x gonadal dysgenesis".ti,ab,kw,kf.

49. trisomy 18 syndrome/

50. trisom* 18 syndrome*.ti,ab,kw,kf.

51. (chromosome* 18 or chromosome* eighteen).ti,ab,kw,kf.

52. edward* syndrome*.ti,ab,kw,kf.

53. Trisomy 13 Syndrome/

54. trisom* 13 syndrome*.ti,ab,kw,kf.

55. (chromosome* 13 or chromosome* thirteen).ti,ab,kw,kf.

56. ((patau* or Bartholin-Patau or Bartholin Patau) adj1 syndrome*).ti,ab,kw,kf.

57. trisom* 21 syndrome*.ti,ab,kw,kf.

58. (chromosome* 21 or chromosome* twenty one or chromosome* twenty-one).ti,ab,kw,kf.

59. ((polysomy or polysomies or tetrasomy or tetrasomies or pentasomy or pentasomies) adj1 (x or y)).ti,ab,kw,kf.

60. (chromosom* adj2 (abnormal* or disorder* or aberration*)).ti,ab,kw,kf.

61. Phelan-McDermid syndrome*.ti,ab,kw,kf.

62. 22q13*.ti,ab,kw,kf.

63. ((cat eye or cat cry) adj1 syndrome*).ti,ab,kw,kf.

64. ((cat-eye or cat-cry) adj1 syndrome*).ti,ab,kw,kf.

65. trisom* 22 syndrome*.ti,ab,kw,kf.

66. (chromosome* 22 or chromosome* twenty two or chromosome* twenty-two).ti,ab,kw,kf.

67. ((DiGeorge* or di george) adj1 syndrome*).ti,ab,kw,kf.

68. 22q11*.ti,ab,kw,kf.

69. ((angelman or beckwith-wiedemann or "beckwith wiedemann" or cri-du-chat or "de lange" or delange or prader-willi or "prader willi" or "fragile x" or fragile-x or rubinstein-taybi or "rubinstein taybi" or orofaciodigital or silver-russell or "silver russell" or smith-magenis or "smith magenis" or sotos or WAGR or williams or wolf-hirschhorn or "wolf hirschhorn") adj2 syndrome*).ti,ab,kw,kf.

70. (tetrasom* or pentasom* or monosom* or disom*).ti,ab,kw,kf.

71. Kleefstra* syndrome*.ti,ab,kw,kf.

72. Alfi* syndrome*.ti,ab,kw,kf.

73. (abnormal* adj1 karyotyp*).ti,ab,kw,kf.

74. (triple* adj1 (x or y) adj1 syndrome*).ti,ab,kw,kf.

75. or/30-74

76. 29 and 75

77. limit 76 to english language

78. limit 77 to yr="2007 -Current"

Appendix B. Additional Methods

Risk of Bias Assessment: Randomized Controlled Trials

Domain	Domain Elements The elements included in each domain are assessed and rated as *Yes*, *No*, *Unclear*, or *Not Applicable* based on performance and documentation of the individual elements in each domain. The overall risk of bias for the study is assessed as *High*, *Moderate*, or *Low* based on assessment of how well the overall study methods and processes were performed to limit bias and ensure validity.
Randomization	• An appropriate method of randomization is used to allocate participants or clusters to groups, such as a computer random number generator • Baseline characteristics between groups or clusters are similar
Allocation Concealment	• An adequate concealment method is used to prevent investigators and participants from influencing enrollment or intervention allocation
Intervention	• Intervention and comparator intervention applied equally to groups • Co-interventions appropriate and applied equally to groups • Control selected is an appropriate intervention
Outcomes	• Outcomes are measured using valid and reliable measures • Investigators use single outcome measures and do not rely on composite outcomes, or the outcome of interest can be calculated from the composite outcome • The trial has an appropriate length of follow-up and groups are assessed at the same time points • Outcome reporting of entire group or subgroups is not selective
Masking (Blinding) of Investigators and Participants	• Investigators and participants are unaware (masked or blinded) of intervention status
Masking (Blinding) of Outcome Assessors	• Outcome assessors are unaware (masked or blinded) of intervention status
Intention to Treat Analysis	• Participants are analyzed based on random assignment (intention-to-treat analysis)
Statistical Analysis	• Participants lost to follow-up unlikely to significantly bias the results (i.e., complete follow-up of ≥ 80% of the participants overall and nondifferential, ≤ 10% difference between groups) • The most appropriate summary estimate (e.g., risk ratio, hazard ratio) is used • Paired or conditional analysis used for crossover RCT • Clustering appropriately accounted for in a cluster-randomized trial (e.g., use of an intraclass correlation coefficient)

Other Biases (as appropriate)	• List others in table footnote and describe, such as:
	  ○ Sample size adequacy
	  ○ Interim analysis or early stopping
	  ○ Recruitment bias, including run-in period used inappropriately
	  ○ Use of unsuitable crossover intervention in a crossover RCT

Interest Disclosure	• Disclosures of interest are provided for authors/funders/commissioners of the study • Interests are unlikely to significantly affect study validity

Domain	Domain Elements The elements included in each domain are assessed and rated as *Yes*, *No*, *Unclear*, or *Not Applicable* based on performance and documentation of the individual elements in each domain. The overall risk of bias for the study is assessed as *High*, *Moderate,* or *Low* based on assessment of how well the overall study methods and processes were performed to limit bias and ensure validity.
Funding	• There is a description of source(s) of funding • Funding source is unlikely to have a significant impact on study validity

Abbreviation. RCT: randomized controlled trial.

Risk of Bias Assessment: Diagnostic Test Accuracy Studies

Domain	Domain Elements The elements included in each domain are assessed and rated as *Yes*, *No*, *Unclear*, or *Not Applicable* based on performance and documentation of the individual elements in each domain. The overall risk of bias for the study is assessed as *High*, *Moderate*, or *Low* based on assessment of how well the overall study methods and processes were performed to limit bias and ensure validity.
Patient Representation	• The spectrum of patients is representative of the patients who will receive the test in practice • The index test, its use, and interpretation are similar to the review question
Patient Selection	• Selection criteria are clearly described • A consecutive or random sample of patients were enrolled • A case-control design was not used • The study avoided inappropriate exclusions
Reference Standard	• The reference standard is likely to classify the condition correctly
Test Timing	• The period between the reference standard and index test is short enough to be reasonably sure that the target condition did not change between the 2 tests
Verification	• The whole sample, or a random selection of the sample, received verification using the same diagnostic reference standard
Use of Reference Standard	• All patients received the same reference standard, regardless of the index test result
Test Independence	• The reference standard was independent of the index test (i.e., the index test did not form part of the reference standard)
Interpretation of the Index Test	• Index test results were interpreted without knowledge of the results of the reference standard • If a threshold was used, it was pre-specified
Interpretation of the Reference Standard	• Reference standard results were interpreted without knowledge of the results of the index test
Uninterpretable or Intermediate Test Results	• Uninterpretable or intermediate test results are reported
Withdrawals	• All patients enrolled were included in the analysis • An explanation is provided for all withdrawals or losses from the study
Interest Disclosure	• Disclosures of interest are provided for authors/funders/commissioners of the study • Interests are unlikely to significantly affect study validity
Funding Source	• There is a description of source(s) of funding • Funding source is unlikely to have a significant impact on study validity

Risk of Bias Assessment: Economic Studies

Domain	Domain Elements The elements included in each domain are assessed and rated as *Yes*, *No*, *Unclear*, or *Not Applicable* based on performance and documentation of the individual elements in each domain. The overall risk of bias for the study is assessed as *High*, *Moderate*, or *Low* based on assessment of how well the overall study methods and processes were performed to limit bias and ensure validity.
Target Population	• Target population and care setting described • Describe and justify basis for any target population stratification, identify any a priori identifiable subgroups • If no subgroup analyses were performed, justify why they were not required
Perspective	• State and justify the analytic perspective (e.g., societal, payer, etc.)
Time Horizon	• Describe and justify the time horizon(s) used in the analysis
Discount Rate	• State and justify the discount rate used for costs and outcomes
Comparators	• Describe and justify selected comparators • Competing alternatives appropriate and clearly described
Modelling	• Model structure (e.g., scope, assumptions made) is described and justified • Model diagram provided, if appropriate • Model validation is described (may involve validation of different aspects such as structure, data, assumptions, and coding and different validation models such as comparison with other models) • Data sources listed and assumptions for use justified • Statistical analyses are described
Effectiveness	• Estimates of efficacy/effectiveness of interventions are described and justified • The factors that are likely to have an impact on effectiveness (e.g., adherence, diagnostic accuracy, values, and preferences) are described and an explanation of how they were factored into the analysis is included • The quality of evidence for the relationship between the intervention and outcomes, and any necessary links, is described
Outcomes	• All relevant outcomes are identified, measured, and valued appropriately (including harms/adverse events) for each intervention, and the justification for information/assumptions is given • Any quality of life measures used in modelling are described and their use justified • Any other outcomes that were considered, but rejected, are described with the rationale for rejection • Ethical and equity-related outcomes are considered and included when appropriate
Resource Use/Costs	• All resources used are identified, valued appropriately, and included in the analyses • Methods for costing are reporting (e.g., patient level) • Resource quantities and unit costs are both reported • Methods for costing time (e.g., lost time, productivity losses) are appropriate and a justification is provided if time costs are not considered
Uncertainty	• Sources of uncertainty in the analyses are identified and justification for probability distributions used in probabilistic analyses are given • For scenario analyses, the values and assumptions tested are provided and justified

Domain	Domain Elements The elements included in each domain are assessed and rated as *Yes*, *No*, *Unclear*, or *Not Applicable* based on performance and documentation of the individual elements in each domain. The overall risk of bias for the study is assessed as *High*, *Moderate*, or *Low* based on assessment of how well the overall study methods and processes were performed to limit bias and ensure validity.
Results	• All results are presented in a disaggregated fashion, by component, in addition to an aggregated manner • All results are presented with undiscounted totals prior to discounting and aggregation • Natural units are presented along with alternative units (e.g., QALYs) • The components of the incremental cost-effectiveness ratio (ICER) are shown (e.g., mean costs of each intervention in numerator and mean outcomes of each intervention in denominator) • Results of scenario analyses, including variability in factors such as practice patterns and costs, are reported and described in relation to the reference (base) case
Interest Disclosure	• Disclosures of interest are provided for authors/funders/commissioners of the study • Interests are unlikely to significantly affect study validity
Funding Source	• There is a description of source(s) of funding • Funding source is unlikely to have a significant impact on study validity

Abbreviations. ICER: incremental cost-effectiveness ratio; QALY: quality-adjusted life year.

Risk of Bias Assessment: Clinical Practice Guidelines

Domain	Domain Elements Assessment indicates how well the guideline methodology and development process were performed to limit bias and ensure validity for elements in domain (each domain rated as *Good*, *Fair*, or *Poor* overall based on performance and documentation of elements)
Rigor of Development: Evidence	• Systematic literature search that meets quality standards for a systematic review (i.e., comprehensive search strategy with, at a minimum, 2 or more electronic databases) • The criteria used to select evidence for inclusion is clear and appropriate • The strengths and limitations of individual evidence sources is assessed and overall quality of the body of evidence assessed
Rigor of Development: Recommendations	• Methods for developing recommendations clearly described and appropriate • There is an explicit link between recommendations and supporting evidence • The balance of benefits and harms is considered in formulating recommendations • The guideline has been reviewed by external expert peer reviewers • The updating procedure for the guideline is specified in the guideline or related materials (e.g., specialty society website)
Editorial Independence	• There is a description of source(s) of funding and the views of the funder(s) are unlikely to have influenced the content or validity of the guideline • Disclosures of interests for guideline panel members are provided and are unlikely to have a significant impact on the overall validity of the guideline (e.g., a process for members to recuse themselves from participating on recommendations for which they have a significant conflict is provided)
Scope And Purpose	• Objectives specifically described • Health question(s) specifically described • Target population(s) for guideline recommendations is specified (e.g., patients in primary care) and target users for the guideline (e.g., primary care clinicians)
Stakeholder Involvement	• Relevant professional groups represented • Views and preferences of target population(s) sought (e.g. clinicians and patients)
Clarity And Presentation	• Recommendations are specific and unambiguous • Different management options are clearly presented • Key recommendations are easily identifiable
Applicability	• Provides advice and/or tools on how the recommendation(s) can be put into practice • Description of facilitators and barriers to its application • Potential resource implications considered • Criteria for implementation monitoring, audit, and/or performance measures based on the guideline are presented

Appendix C. Evidence Tables

Table C1. Study Characteristics for Randomized Controlled Trials

Citation Setting NCT Number	Study Aim Study Design	Inclusion and Exclusion Criteria	Patient Characteristics	Prenatal Screening	Comparator(s)	Outcomes Measured
Kagan et al., 2018[8] Germany Not reported	To compare risk assessment by cFTS with ultrasound examination at 11–13 weeks' gestation and cfDNA RCT	Inclusion criteria (must meet all): Pregnant women with a normal first-trimester ultrasound examination (fetal NT ≤ 3.5 mm and no fetal defects) Exclusion criteria (excluded if any criteria met): Aged < 18 years; CRL measurement > 84 mm or < 45 mm; multiple pregnancy, including vanishing twins	Total N = 1,400 randomized, with 1,376 included in the analysis Excluded: 13 of 701 (1.9%) in the cfDNA arm (5 miscarriage or IUD; 8 lost to follow-up); 11 of 699 (1.6%) in the cFTS group (3 miscarriage or IUD; 8 lost to follow-up) Median maternal age: 33.9 years (IQR, 31.0 to 36.8) cfDNA; 33.9 years (IQR, 30.7 to 36.7) cFTS Median gestational age: 12.7 weeks (IQR, 12.4 to 13.1) cfDNA; 12.7 weeks (IQR, 12.3 to 13.1) cFTS Median maternal weight: 65.4 kg (IQR, 59.0 to 73.7) cfDNA; 66.0 (IQR, 59.1 to 74.3) cFTS Median maternal BMI: 23.4 kg/m^2 (IQR, 21.2 to 26.1) cfDNA; 23.4 kg/m^2 (IQR, 21.2 to 26.6) cFTS Ethnicity, Caucasian: 672 (97.7%) cfDNA; 676 (98.3%) cFTS Cigarette smoker: 19 (2.8%) cfDNA; 23 (3.3%) cFTS	Harmony Prenatal (Roche-Ariosa) TMPS Ultrasound	cFTS (maternal and gestational age, fetal NT thickness, and maternal levels of serum PAPP-A and free β-hCG)	False-positive rate

Citation Setting NCT Number	Study Aim Study Design	Inclusion and Exclusion Criteria	Patient Characteristics	Prenatal Screening	Comparator(s)	Outcomes Measured
			Assisted reproduction: 44 (6.4%) cfDNA; 29 (4.2%) cFTS			

Abbreviations. BMI: body mass index: cfDNA: cell-free DNA; cFTS: combined FTS; CRL:crown rump length FTS: first-trimester screening; hCG: human chorionic gonadotrophin; IQR: interquartile range; IUD: intrauterine device; NT: nuchal translucency; PAPP-A: pregnancy-associated plasma protein A; RCT: randomized controlled trial; TMPS: targeted massively parallel sequencing.

Table C2. Evidence Tables for Randomized Controlled Trials

Citation Setting NCT Number	Pregnancy and Test Outcomes	Changes in Management	Uptake of Prenatal Screening	Quality of Life
Kagan et al., 2018[8,94,95] Germany Not reported	No pregnancies with T21, T18, or T13 observed Failed cfDNA tests: 10 (1.5%) T21 FP rate: 0, (95% CI, 0% to 0.5%) cfDNA; 2.5% (95% CI, 1.5% to 3.9%) cFTS; *P* < .0001 T18 FP rate: 0, cfDNA; 0, cFTS T13 FP rate: 0, cfDNA; 1 (0.1%), cFTS See Tables C3 and C4 for risk distributions When other ultrasound markers (nasal bone assessment and Doppler evaluation of the tricuspid valve and ductus venous flow) were included, the rate of T21 FP results remained lower in the cfDNA group compared with the extended cFTS. The differences were not statistically significant.	Invasive testing: 2 (0.3%) cfDNA; 12 (1.7%) cFTS Women in the cfDNA group chose invasive testing based on a personal risk for trisomy or personal choice cFTS: 6 of 17 (35.3%) women with high risk T21 opted for invasive testing; 9 of 17 (52.9%) for additional cfDNA testing; 2 (11.8%) decided against any further evaluation cFTS: 6 low risk women decided to undergo invasive testing	Not reported	Not reported

Abbreviations. cfDNA: cell-free DNA; cFTS: combined FTS; CI: confidence interval; FP: false positive; FTS: first-trimester screen; T13: trisomy 13, T18: trisomy 18; T21: trisomy 21.
Note. Results from the retrospective cohort have not been reported as it included women at high-risk.

Table C3. Risk Distributions (Kagan et al., 2018[8])

Risk Distribution	cfDNA + Ultrasound	cFTS	*P* Value
Median risk for T21	1 in 10,000 (IQR, 10,000 to 10,000)	1 in 3,787 (IQR, 1,605 to 8,280)	Not reported
T21 risk > 1:100	0 (0%)	17 (2.5%)	< .0001
T21 risk 1:100 to 1:999	2 (0.3%)	79 (11.5%)	Not reported
T21 risk 1:1,000 to 1:4,999	1 (0.1%)	302 (43.9%)	Not reported
T21 risk 1:5,000 to 1:9,999	4 (0.6%)	163 (23.7%)	Not reported
T21 risk ≤ 1:10,000	681 (99.0%)	127 (18.5%)	Not reported

Abbreviations. cfDNA: cell-free DNA; cFTS: combined first-trimester screening; IQR: interquartile range; T21: trisomy 21. Source. Adapted from Kagan et al., 2018.[8]

Table C4. Risk for Trisomy in Euploid Fetuses (Kagan et al., 2018[8,94])

Risk for Trisomy in Euploid Fetuses	cfDNA + Ultrasound			cFTS		
	T21	T18	T13	T21	T18	T13
< 1:10	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.1%)
1:10 to 1:99	0 (0%)	0 (0%)	0 (0%)	17 (2.5%)	3 (0.4%)	0 (0%)
1:100 to 1:999	2 (0.3%)	1 (0.1%)	0 (0%)	79 (11.5%)	14 (2.0%)	6 (0.9%)
1:1,000 to 1:9,999	5 (0.7%)	3 (0.4%)	2 (0.3%)	465 (67.6%)	222 (32.3%)	80 (11.6%)
≤ 1:10,0000	681 (99.0%)	684 (99.4%)	686 (99.7%)	127 (18.5%)	449 (65.3%)	601 (87.4%)
FP rate	0 (0%)	0 (0%)	0 (0%)	17 (2.5%)	0 (0%)	0 (0%)
Overall FP rate for T21, T18, and T13	0 (0%)			17 (2.5%)		

Abbreviations. cfDNA: cell-free DNA; cFTS: combined first-trimester screening; FP: false positive. T13: trisomy 13; T18; trisomy 18; T21: trisomy 21. Source. Adapted from Kagan et al., 2018.[8,94]

Table C5. Study Characteristics for Test Accuracy Studies

Citation Setting NCT Number	Study Aim Study Design	Inclusion and Exclusion Criteria	Prior Risk	Patient Characteristics	Index Test(s) and Reference Standard	Target Conditions
Singleton Pregnancies						
Ashoor et al., 2013[9] U.K. and U.S. Not reported	To assess the performance of chromosome-selective sequencing of maternal plasma cfDNA in non-invasive prenatal testing for T13	Inclusion criteria (must meet all): Pregnant women, singleton pregnancies, underwent routine cFTS and subsequently delivered	Low risk (phase 2 participants only)	Total N = 2,002, with 1,949 included in the main analysis Euploid pregnancies n = 1,939 T13 pregnancies n = 10 (cases from the U.S., not the selected population)	Harmony Prenatal (Roche-Ariosa) TMPS Birth outcomes	T13

Citation Setting NCT Number	Study Aim Study Design	Inclusion and Exclusion Criteria	Prior Risk	Patient Characteristics	Index Test(s) and Reference Standard	Target Conditions
	Prospective cohort and case-controlled	phenotypically normal neonates Also included 13 confirmed cases of T13 (no details reported) Exclusion criteria (excluded if any criteria met): Not reported		Excluded: 53 (2.6%) failed amplification and sequencing Median maternal age: 31.8 years (SD, 5.6) euploid; 37.5 years (SD, 5.3) T13 Median gestational age: 12.6 weeks (SD, 0.56) euploid; 20.9 weeks (SD, 3.88) T13 Race or ethnicity: for euploid pregnancies, 1,370 (70.7%) Caucasian, 387 (20.0%) African, 131 (6.8%) Asian, 51 (2.6%) mixed; 8 (80.0%) for T13 pregnancies, Caucasian, 2 (20.0%) African, 0 Asian, 0 mixed Median fetal fraction: 10.0% euploid (range, 4.1% to 31.0%); 14.0% T13 (range, 6.1 to 24.0%)		
Bianchi et al., 2014[10] U.S. NCT01663350	To compare noninvasive prenatal cfDNA testing for fetal autosomal aneuploidy with conventional screening in a general obstetrical population Prospective cohort	Inclusion criteria (must meet all): Pregnant women, at least 18 years of age, gestational age of at least 8 weeks, singleton pregnancy, planned to undergo or completed standard screening Exclusion criteria (excluded if any criteria met): Not reported	Low risk, described as a general obstetric population undergoing standard prenatal screening	Total N = 2,042, with 1,914 included in the main analysis T21, N = 1,909 T18, N = 1,905 Excluded: 72 of 2,042 (3.5%) no clinical outcome, 48 of 2,042 (2.4%) lost to follow-up, 24 of 2,042 (1.2%) no live birth and no karyotype, 17 of 2,042 (0.8%) no cfDNA result, 39 of 2,042 (1.9%) no result for standard screening	verifi (Illumina) MPSS Standard screening Birth outcomes or karyotyping	T21 T18

Citation Setting NCT Number	Study Aim Study Design	Inclusion and Exclusion Criteria	Prior Risk	Patient Characteristics	Index Test(s) and Reference Standard	Target Conditions
				Mean maternal age: 29.6 years (range, 18.0 to 48.6) Ethnicity: 213 (11.1%) Hispanic or Latino, 1 (0.1%) unknown Race or ethnicity: 1,252 (65.4%) White, 427 (22.3%) Black, 140 (7.3%) Asian, 16 (0.8%) American Indian or Alaska Native, 16 (0.8%) Native Hawaiian or other Pacific Islander, 63 (3.3%) multiracial or other Mean BMI: 28.7 kg/m^2 (range, 15.5 to 59.0) Maternal medical history: 38 (2.0%) diabetes mellitus, 72 (3.8%) hypothyroidism, 9 (0.5%) hyperthyroidism, 19 (1.0%) other autoimmune disorder, 23 (1.2%) thrombophilia First pregnancy: 1,299 (67.9%) Pregnancy by ART: 66 (3.4%) Mean gestational age at time of testing: 20.3 weeks (range, 8.0 to 39.4) Pregnancy trimester at time of cfDNA testing: 759 (39.7%) first (< 14 weeks' gestation), 610 (31.9%) second (14 weeks to < 27 weeks), 545 (28.5%) third (27 weeks or more)		

Citation Setting NCT Number	Study Aim Study Design	Inclusion and Exclusion Criteria	Prior Risk	Patient Characteristics	Index Test(s) and Reference Standard	Target Conditions
				Type of standard screening[a]: 739 (38.6%) first-trimester combined,[b] 519 (27.1%) sequential,[c] 53 (2.8%) fully integrated,[d] 164 (8.6%) serum integrated,[e] 439 (22.9%) second-trimester quadruple[f]		
Langlois et al., 2017[12] Canada NCT01925742	To evaluate the impact of offering cfDNA screening as a first-tier test for T21 and T18 Prospective cohort	Inclusion criteria (must meet all): Pregnant women aged 19 years or older; who have a singleton gestation; are recruited before 14 weeks' gestation; decided to undertake the provincially funded screening test, serum integrated screen, or integrated prenatal screen; agreed to have the cfDNA screening result provided at the same time as the result of their standard screen Exclusion criteria (excluded if any criteria met): Not reported	Low risk (no details provided)	Total N = 1,198, with 1,165 included in the main analysis Excluded: 14 (1.2%) lost to follow-up, 1 termination, 2 (0.2%) fetal anomalies with no karyotyping, 12 (1.0%) spontaneous abortion before screening complete, 3 (0.3%) wrong gestational dating, 1 (0.1%) stillbirth with no chromosomal analysis Mean maternal age at expected delivery date: 33.2 years (range, 19 to 46) Mean maternal weight: 65.7 kg (range, 39.9 to 167) Mean gestational age at time of cfDNA blood draw: 12.1 weeks (range, 10 to 13.9) Type of standard screening: 287 (24.6%) cFTS (first-trimester PAPP-A and free β-hCG, and NT); 493 (42.3%) SIPS, 374 (32.1%) IPS, 11 (0.9%) 2T QUAD	Harmony Prenatal (Roche-Ariosa) TMPS Standard screening, with cFTS, SIPS, IPS, or 2T QUAD Birth outcomes or karyotyping	T21 T18 T13
Nicolaides et al., 2012[13]	To assess the performance of cfDNA screen tests for	Inclusion criteria (must meet all):	Low risk, described as a	Total N = 2,230, with 2,049 included in the main analysis	Harmony Prenatal (Roche-Ariosa)	T21

Citation Setting NCT Number	Study Aim Study Design	Inclusion and Exclusion Criteria	Prior Risk	Patient Characteristics	Index Test(s) and Reference Standard	Target Conditions
U.K. Not reported	fetal trisomy in a routinely screened first-trimester pregnancy population Prospective cohort	Pregnant women with a singleton pregnancy attending their first routine hospital visit Exclusion criteria (excluded if any criteria met): Not reported	general pregnancy population	Excluded: 28 (1.3%) no fetal karyotype and pregnancy outcome of miscarriage, stillbirth or termination, 46 (2.1%) no follow-up, 7 (0.3%) abnormal fetal karyotype not of interest, 29 (1.3%) inadequate sample volume at testing, 1 (0.04%) label mismatch, 70 (3.14%) sample mixing issue Median maternal age: 31.8 years (IQR, 27.7 to 35.4) Median maternal weight: 65.2 kg (IQR, 58.5 to 76.0) Median maternal height: 164 cm (IQR, 160 to 169) Race or ethnicity: 1,431 (69.8%) Caucasian, 422 (20.6%) African, 82 (4.0%) South Asian, 57 (2.8%) East Asian, 57 (2.8%) mixed Cigarette smoker: 131 (6.4%) Method of conception: 2,007 (98.0%) spontaneous, 19 (0.9%) ovulation drugs, 23 (1.1%) IVF Preexisting diabetes: 10 (0.5%) type 1, 9 (0.4%) type 2	TMPS FTS, comprising serum measurement of PAPP-A and free ß-hCG with NT measurement Birth outcomes or karyotyping	T18
Norton et al., 2015[14]	To determine whether cfDNA testing has better	Inclusion criteria (must meet all):	Low risk, described as a	Total N = 18,955, with 15,841 included in the main analysis	Harmony Prenatal (Roche-Ariosa)	T21

Citation Setting NCT Number	Study Aim Study Design	Inclusion and Exclusion Criteria	Prior Risk	Patient Characteristics	Index Test(s) and Reference Standard	Target Conditions
U.S., Belgium, Canada, Italy, Netherlands, and Sweden NCT01511458	performance than standard FTS in risk assessment for T21, T18, and T13 in a large, unselected population of women presenting for aneuploidy screening Prospective cohort	Women at least 18 years of age, singleton pregnancy between 10.0 and 14.3 weeks Exclusion criteria (excluded if any criteria met): Outside the gestational-age window, no standard screening result, known maternal aneuploidy or cancer, conceived with the use of donor oocytes, twin pregnancy, empty gestational sac identified on ultrasound	large, unselected population of women presenting for aneuploidy screening	Excluded: 229 (1.2%) as not eligible, 31 (0.2%) with twins, 121 (0.6%) unknown ovum-donor status, 64 (0.3%) withdrew or were withdrawn by the investigator, 384 (2.0%) sample handling errors, 308 (1.6%) no standard screening results, 488 (2.6%) no cfDNA result, 1,489 (7.9%) lost to follow-up Mean maternal age: 31 years (range, 18 to 48) Mean gestational age at sample collection: 12.5 weeks (range, 10.0 to 14.3) Race or ethnicity: 11,235 (70.9%) White, 1,295 (8.2%) Black, 1,659 (10.5%) Asian, 93 (0.6%) Native American, 422 (2.7%) multiracial, 1,060 (6.7%) other, 77 (0.5%) not reported Hispanic ethnic group: 3,202 (20.2%) Hispanic, 12,639 (79.8%) non-Hispanic Median maternal weight: 65.8 kg (range, 31.8 to 172.4) Pregnancy through ART: 480 (3.0%)	TMPS Standard screening (including PAPP-A, total or free ß subunit of hCG, and NT Birth outcomes and genetic testing	T18 T13

Citation Setting NCT Number	Study Aim Study Design	Inclusion and Exclusion Criteria	Prior Risk	Patient Characteristics	Index Test(s) and Reference Standard	Target Conditions
				Current smoker: 432 (2.7%) Insulin-dependent diabetes: 188 (1.2%)		
Pergament et al., 2014[16] U.S., Czech Republic, Japan, Turkey, Ireland, Spain, and Poland Not reported	To estimate performance of a SNP–based noninvasive prenatal screen for fetal aneuploidy in high-risk and low-risk populations Prospective cohort	Inclusion criteria (must meet all): Women at least 18 years of age, singleton pregnancy at 7 weeks or later Exclusion criteria (excluded if any criteria met): Not reported	Mixed-risk population	Total N = 1,064, with 1,051 included in the main analysis Excluded: 6 confirmed triploidy; 3 fetal mosaic; 2 47,XXY; 1 47,XXX; 1 47,XYY Mean maternal age: 30.3 years (range, 18 to 47) Mean gestational age: 17.0 (range, 7.6 to 40.6) High risk, defined after positive serum screen, ultrasound abnormality, and/or maternal age of ≥35 years: 543 (51.0%) Low risk, defined as maternal age of <35 years and lacking any reported high-risk indications: 521 (49.0%)	Panorama (Natera) TMPS Invasive testing	T21 T18 T13 45,X
Quezada et al., 2015[17] U.K. Not reported	To examine in a general population the performance of cfDNA testing for trisomies 21, 18, and 13 at 10–11 weeks' gestation and compare it to that of the combined test at 11–13 weeks Prospective cohort	Inclusion criteria (must meet all): Women with singleton pregnancies Exclusion criteria (excluded if any criteria met): Not reported	Low risk (described as a general population)	Total N = 2,905, with 2,785 included in main analysis Excluded: 120 (4.1%) unknown trisomic status Median maternal age: 36.9 years (range, 20.4 to 51.9)	Harmony Prenatal (Roche-Ariosa) TMPS Invasive testing and birth outcomes	T21 T18 T13

Citation Setting NCT Number	Study Aim Study Design	Inclusion and Exclusion Criteria	Prior Risk	Patient Characteristics	Index Test(s) and Reference Standard	Target Conditions
				Aged 35 and older: 1,958 of 2,905 (67.4%) Race or ethnicity: 2,570 (88.5%) Caucasian, 173 (6.0%) South Asian, 96 (3.3%) East Asian, 21 (0.7%) Afro-Caribbean, 45 (1.5%) mixed Parity: 1,555 (53.5%) parous; 1,350 (46.5%) nulliparous Conception: 2,438 (83.9%) spontaneous; 467 (16.1%) ART		
Twin Pregnancies						
del Mar Gil et al., 2014[11] U.K. Not reported	To examine the clinical implementation of chromosome-selective sequencing of cfDNA in maternal blood in the assessment of risk for trisomies in twin pregnancies Retrospective cohort (stored samples)	Inclusion criteria (must meet all): Women undergoing cFTS, twin pregnancies Exclusion criteria (excluded if any criteria met): Not reported	Low risk (all undergoing cFTS)	Total N = 207, with 192 included in the main analysis Excluded: 15 (7.2%) no cfDNA results (11 low fetal fraction, 4 laboratory processing issues) Birth outcome: 193 (93.2%) with 10 (4.8%) T21, 1 (0.5%) T18, and 3 (1.4%) T13 Median maternal age: 33.6 years (IQR, 29.0 to 36.6) euploid; 36.7 years (IQR, 34.2 to 37.9) T21; 41.0 years T18; 28.3 years (IQR, 26.7 to 34.5) Median maternal weight: 67.0 kg (IQR, 60.5 to 78.0) euploid; 69.5 kg (IQR, 62.5 to 73.2) T21; 65.0 kg T18; 71.0 kg (IQR, 65.5 to 78.4) T13	Harmony Prenatal (Roche-Ariosa) TMPS Fetal karyotyping	T21 T18 T13

Citation Setting NCT Number	Study Aim Study Design	Inclusion and Exclusion Criteria	Prior Risk	Patient Characteristics	Index Test(s) and Reference Standard	Target Conditions
Mixed Singleton and Twin Pregnancies						
Palomaki et al., 2017[15,92] U.S. NCT01966991	To assess the clinical utility of cfDNA-based screening for aneuploidies offered through primary obstetrical care providers to a general pregnancy population Prospective cohort	Inclusion criteria (must meet all): Women at least 18 years of age; singleton pregnancy at 10 weeks or later, eligible and opting for cfDNA screening Exclusion criteria (excluded if any criteria met): Screen positive DNAFirst result	Low risk, described as a general pregnancy population	Total N = 2,691, with 2,681 included in the main analysis Excluded: 6 (0.2%) no initial test and never retested, 3 (0.1%) unknown twins, 1 (0.04%) donated egg Median week of testing (n = 2,685): week 12 (range, 9 to 31) Sampled after 20 weeks (n = 2,685): 43 (1.6%) Dating performed by ultrasound (n = 2,685): 2,421 (90%) Median maternal age (n = 2,685): 31 years (range, 14 to 45) Maternal age 35 or older (n = 2,685): 564 (21%) Median maternal weight (n = 2,513): 68 kg (range, 37 to 167) Median maternal height (n = 2,101): 1.63 m (range, 1.35 to 1.93) Median maternal BMI (n = 2,071): 25.5 kg/m^2 (range, 14.6 to 54.7)	Panorama (Natera) SNP TMPS Invasive testing Diagnostic testing (e.g., karyotyping) Birth outcomes	T21 T18 T13 45,X

Citation Setting NCT Number	Study Aim Study Design	Inclusion and Exclusion Criteria	Prior Risk	Patient Characteristics	Index Test(s) and Reference Standard	Target Conditions
				Insulin-dependent diabetes (n = 2,681): 11 (0.4%) Cigarette smoker (n = 2,597): 69 (2.7%) Race or ethnicity (n = 2,266): 1,934 (85%) Caucasian, 142 (6%) African American, 96 (4%) Asian American, 94 (4%) other Hispanic ethnicity (n = 2,489): 343 (14%) Indication for testing (n = 2,685): 2,371 (88%) routine screen, 260 (10%) AMA, 27 (1%) history of spontaneous fetal loss, 9 (< 1%) history of chromosome abnormality, 6 (< 1%) abnormal ultrasound, 2 (< 1%) abnormal serum screen, 10 (< 1%) other		

Abbreviations. 2T QUAD: second-trimester quadruple screening; 45,X: Turner syndrome; 47,XXX: Triple X syndrome; 47,XXY: Klinefelter syndrome; 47,XYY: Jacob's syndrome; AFP: alpha-fetoprotein; AMA: advanced maternal age; ART: assisted reproductive techniques; BMI: body mass index; cfDNA: cell-free DNA; cFTS: combined FTS; FTS: first-trimester screening; hCG: human chorionic gonadotrophin; IPS: integrated screening with SIPS and NT; IQR: interquartile range; IVF: in vitro fertilization; MPSS: massively parallel shotgun sequencing; NT: nuchal translucency; PAPP-A: pregnancy-associated plasma protein A; SD: standard deviation; SIPS: first-trimester PAPP-A, second-trimester AFP, hCG, uE3, inhibin A; SNP: single nucleotide polymorphism; T13: trisomy 13; T18: trisomy 18; T21: trisomy 21; TMPS: targeted massively parallel sequencing; uE3: unconjugated estriol 3. Note. [a] First-trimester serum markers were pregnancy-associated plasma protein A and free beta subunit or total hCG, and second-trimester serum markers were maternal serum AFP, hCG, unconjugated estriol, and inhibin A; [b] First-trimester serum markers combined with fetal nuchal translucency; [c] Results of the first-trimester screening were reported before the final report in the second trimester; [d] First-trimester and second-trimester results combined, including serum markers and NT; [e] First-trimester and second-trimester results combined if the first trimester only included serum markers; [f] Second-trimester serum markers evaluated alone.

Table C6. Evidence Tables for Test Accuracy Studies

<table>
<tr><th>Citation
Setting
NCT Number</th><th>Test Results</th><th>Test Performance</th><th>Test Failures</th><th>Pregnancy and Other Outcomes</th></tr>
<tr><td colspan="5">Singleton Pregnancies</td></tr>
<tr>
<td>Ashoor et al., 2013[9]

U.K. and U.S.

Not reported</td>
<td>T13
cfDNA vs. reference standard
<table>
<tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr>
<tr><td>8</td><td>1</td><td>2</td><td>1,938</td></tr>
</table>
See also Nicolaides et al., 2012[13] for T21 and T18 results and all trisomies combined</td>
<td>T13
cfDNA vs. reference standard

Sensitivity: 80.0% (95% CI, 44.4% to 97.4%)
Specificity: 99.9% (95% CI, 99.7% to 100%)

Condition prevalence: 0.5% (95% CI, 0.2% to 0.9%)

PPV: 88.9% (95% CI, 52.4% to 98.3%)
NPV: 99.9% (95% CI, 99.6% to 99.97%)

Accuracy: 99.8% (95% CI, 99.6% to 99.97%)</td>
<td>Test failure at amplification and sequencing: 53 of 2,002 (2.6%)</td>
<td>Not reported</td>
</tr>
<tr>
<td>Bianchi et al., 2014[10]

U.S.

NCT01663350</td>
<td>T21
cfDNA vs. reference standard
<table>
<tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr>
<tr><td>5</td><td>6</td><td>0</td><td>1,941</td></tr>
</table>
T21
Standard screening vs. reference standard
<table>
<tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr>
<tr><td>3</td><td>69</td><td>0</td><td>1,840</td></tr>
</table>
T18
cfDNA vs. reference standard
<table>
<tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr>
</table>
</td>
<td>Tests in all trimesters:
T21
cfDNA vs. reference standard

Sensitivity: 100% (95% CI, 47.8% to 100%)
Specificity: 99.7% (95% CI, 99.3% to 99.9%)

Condition prevalence: 0.3% (95% CI, 0.1% to 0.6%)

PPV: 45.4% (95% CI, 27.3% to 64.9%)[a]
NPV: 100%</td>
<td>cfDNA
18 of 2,042 (0.9%), with approximately half of these during extraction and half during sequencing; no clear biologic reasons for failures

Standard screening
4 of 2,042 (0.2%) reported as uninterpretable

Outcomes for patients with failed or uninterpretable tests were not reported.</td>
<td>Patients who had positive cfDNA screens and negative standard screening results had live births with normal physical examinations

Seventeen patients with positive results on standard screening underwent invasive prenatal procedures and 27 patients with negative results on standard screening elected to undergo an invasive prenatal procedure (CVS, 5; amniocentesis, 22).</td>
</tr>
</table>

<table>
<tr><th>Citation
Setting
NCT Number</th><th>Test Results</th><th>Test Performance</th><th>Test Failures</th><th>Pregnancy and Other Outcomes</th></tr>
<tr>
<td></td>
<td>
<table>
<tr><td>2</td><td>3</td><td>0</td><td>1,947</td></tr>
</table>
T18
Standard screening vs. reference standard
<table>
<tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr>
<tr><td>1</td><td>11</td><td>0</td><td>1,894</td></tr>
</table>
T13
cfDNA vs. reference standard
<table>
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<tr><td>1</td><td>3</td><td>0</td><td>1,910</td></tr>
</table>
T13
Standard screening vs. reference standard
<table>
<tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr>
<tr><td>1</td><td>6</td><td>0</td><td>892</td></tr>
</table>
All trisomies (T21, T18, T13)
cfDNA vs. reference standard
<table>
<tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr>
<tr><td>8</td><td>12</td><td>0</td><td>1,932</td></tr>
</table>
All trisomies (T21, T18, T13)
Standard screening vs. reference standard
Not calculable
</td>
<td>
Accuracy: 99.7% (95% CI, 99.3% to 99.9%)

T21
Standard screening vs. reference standard
Sensitivity: 100% (95% CI, 29.2% to 100%)
Specificity: 96.4% (95% CI, 95.5% to 97.2%)

Condition prevalence: 0.2% (95% CI, 0.03% to 0.5%)

PPV: 4.17% (95% CI, 3.3% to 5.2%)[a]
NPV: 100%

Accuracy: 96.4% (95% CI, 95.4% to 97.2%)[a]

T18
cfDNA vs. reference standard
Sensitivity: 100.00% (95% CI, 15.8% to 100.00%)
Specificity: 99.8% (95% CI, 99.5% to 99.97%)

Condition prevalence: 0.1% (95% CI, 0.01% to 0.4%)

PPV: 40.0% (95% CI, 17.7% to 67.4%)[a]
NPV: 100%
</td>
<td></td>
<td>All fetal karyotypes were normal, and all results of cfDNA testing were negative for T21, T18, and T13.</td>
</tr>
</table>

Citation Setting NCT Number	Test Results	Test Performance	Test Failures	Pregnancy and Other Outcomes
		Accuracy: 99.8% (95% CI, 99.6% to 99.97%) **T18** **Standard screening vs. reference standard** Sensitivity: 100% (95% CI, 2.5% to 100%) Specificity: 99.4% (95% CI, 99.0% to 99.7%) Condition prevalence: 0.05% (95% CI, 0% to 0.3%)[a] PPV: 8.3% (95% CI, 4.8% to 14.1%)[a] NPV: 100% Accuracy: 99.4% (95% CI, 99.0% to 99.7%) **T13** **cfDNA vs. reference standard** Sensitivity: 100% (95% CI, 2.5% to 100%) Specificity: 99.8% (95% CI, 99.5% to 99.97%) Condition prevalence: 0.05% (95% CI, 0% to 0.3%) PPV: 25.0% (95% CI, 9.7% to 50.8%)[a] NPV: 100%		

Citation Setting NCT Number	Test Results	Test Performance	Test Failures	Pregnancy and Other Outcomes
		Accuracy: 99.8% (95% CI, 99.5% to 99.97%) **T13** **Standard screening vs. reference standard** Sensitivity: 100% (95% CI, 2.5% to 100%) Specificity: 99.3% (95% CI, 98.6% to 99.8%) Condition prevalence: 0.1% (95% CI, 0% to 0.6%) PPV: 14.3% (95% CI, 7.0% to 27.0%)[b] NPV: 100% Accuracy: 99.3% (95% CI, 98.5% to 99.7%) **All trisomies (T21, T18, T13)** **cfDNA vs. reference standard** Sensitivity: 100.00% (95% CI, 63.1% to 100.00%) Specificity: 99.4% (95% CI, 98.9% to 99.7%) Condition prevalence: 0.4% (95% CI, 0.2% to 0.8%) PPV: 40.0% (95% CI, 27.5% to 54.0%)[a] NPV: 100%		

<table>
<tr><th>Citation
Setting
NCT Number</th><th>Test Results</th><th>Test Performance</th><th>Test Failures</th><th>Pregnancy and Other Outcomes</th></tr>
<tr><td></td><td></td><td>Accuracy: 99.4% (95% CI, 98.9% to 99.7%)

Results did not change significantly when the analysis was limited to the subgroup of patients whose blood samples were obtained during the first or second trimester (< 27 weeks of gestational age).

There was no overlap in the women who had FP results between cfDNA and standard screening.

Mean fetal fraction in patients ≥ 35 who had positive results on standard screening or both standard screening and cfDNA screening: 11.3%

Mean fetal fraction in patients < 35 years of age who had negative results on standard screening or both standard screening and cfDNA screening: 11.6%

Mean fetal fraction in patients who provided blood in the third trimester: 24.6%</td><td></td><td></td></tr>
<tr><td>Langlois et al., 2017[12]

Canada</td><td>T21
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>6</td><td>0</td><td>0</td><td>1,159</td></tr></table></td><td>T21
cfDNA vs. reference standard
Sensitivity: 100% (95% CI, 54.1% to 100%)</td><td>No result on first cfDNA blood draw: 11 of 1,165 (0.9%; 95% CI, 0.47% to 1.7%)</td><td>Serum integrated screening identified 4 cases of T18. None had T18 but all had abnormal outcomes.</td></tr>
</table>

<table>
<tr><th>Citation
Setting
NCT Number</th><th>Test Results</th><th>Test Performance</th><th>Test Failures</th><th>Pregnancy and Other Outcomes</th></tr>
<tr>
<td>NCT01925742</td>
<td>
T21
Standard screening vs. reference standard
<table>
<tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr>
<tr><td>5</td><td>63</td><td>1</td><td>1,096</td></tr>
</table>
T18
cfDNA vs. reference standard
<table>
<tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr>
<tr><td>0</td><td>1</td><td>0</td><td>1,164</td></tr>
</table>
T18
Not reported

T13
cfDNA vs. reference standard
<table>
<tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr>
<tr><td>0</td><td>1</td><td>0</td><td>1,164</td></tr>
</table>
T13
Standard screening vs. reference standard
Not reported

All trisomies (T21, T18, T13)
cfDNA vs. reference standard
<table>
<tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr>
<tr><td>6</td><td>2</td><td>0</td><td>1,157</td></tr>
</table>
All trisomies (T21, T18, T13)
Standard screening vs. reference standard
Not reported
</td>
<td>
Specificity: 100% (95% CI, 99.7% to 100%)[a]

Condition prevalence: 0.5% (95% CI, 0.2% to 1.1%)

PPV: 100%
NPV: 100%

Accuracy: 100% (95% CI, 99.7% to 100%)

T21
Standard screening vs. reference standard
Sensitivity: 83.3% (95% CI, 35.9% to 99.6%)
Specificity: 94.6% (95% CI, 93.1% to 95.8%)

Condition prevalence: 0.5% (95% CI, 0.2% to 1.1%)

PPV: 7.4% (95% CI, 4.9% to 10.9%)
NPV: 99.9% (95% CI, 99.5% to 99.98%)

Accuracy: 94.5% (95% CI, 93.0% to 95.7%)

T18
cfDNA vs. reference standard
Sensitivity: not calculable
Specificity: 99.9% (95% CI, 99.5% to 100%)
</td>
<td>
10 low fetal fraction
1 unusually high variance in cfDNA, which failed repeat testing for low fetal fraction

Failure on second cfDNA blood draw, 6 of 11 (54.5%): 3 had major structural anomalies identified through second-trimester ultrasound, with a diagnosis of triploidy;
3 had a negative standard screen and a normal second-trimester ultrasound; all pregnancies had a normal outcome

Mean maternal weight for the 8 women in whom a diagnosis of triploidy was not made: 94 kg (range, 58.5 to 131 kg)
</td>
<td>
One cfDNA negative screen for T21, T18, and T13 had abnormal growth on ultrasound and mosaicism for 46,r(X)(p22.11q23) or 45,X on amniocentesis.

Three conventional screen positives for T18 had 2 failed cfDNA attempts due to a low fetal fraction. Ultrasound detected structural abnormalities in each case, all of which were diagnosed with triploidy.

Two women whose pregnancies were cfDNA positive for T18 and T13 underwent amniocentesis; in each case, the fetus was found to have a normal karyotype. Both births were live and normal.

Seven cases of chromosomal abnormalities other than T21, T18, and T13 were identified; 2 (28.6%) may have been detected with the addition of 45,X or an SCA panel. All cases were detected based on ultrasound anomalies in the second trimester. No additional cases of chromosomal
</td>
</tr>
</table>

<table>
<tr><th>Citation
Setting
NCT Number</th><th>Test Results</th><th>Test Performance</th><th>Test Failures</th><th>Pregnancy and Other Outcomes</th></tr>
<tr><td></td><td></td><td>Condition prevalence: 0% (95% CI, 0% to 0.3%)

PPV: 0%
NPV: 100%

Accuracy: 99.9% (95% CI, 99.5% to 100%)

T18
Standard screening vs. reference standard
FP rate by SIPS: 0 of 1,152 (0%; 95% CI, 0% to 0.3%) (none had T18 but had other abnormal outcomes)

T13
cfDNA vs. reference standard
Sensitivity: not calculable
Specificity: 99.9% (95% CI, 99.5% to 100%)

Condition prevalence: 0% (95% CI, 0% to 0.3%)

PPV: 0%
NPV: 100%

Accuracy: 99.9% (95% CI, 99.5% to 100%)

T13
Standard screening vs. reference standard</td><td></td><td>anomalies were diagnosed postnatally.

23 women underwent invasive diagnostic testing:
<table><tr><th>Indication</th><th>N</th></tr><tr><td>+ve cfDNA</td><td>8</td></tr><tr><td>-ve cfDNA, +ve T21 screen</td><td>3</td></tr><tr><td>Second-trimester ultrasound anomaly</td><td>10</td></tr><tr><td>Fetal sex discrepancy cfDNA and ultrasound</td><td>1</td></tr><tr><td>+ve serum screen for Smith-Lemli-Opitz syndrome</td><td>1</td></tr></table>
Of the 68 women positive for T21 by traditional screen, 6 were positive by cfDNA screening (5 positive for T21, 1 positive for T13) and all underwent invasive diagnostic testing that confirmed T21 in the 5 cases and was normal in the case of the positive cfDNA test for T13.

Overall, 59 women with a positive traditional screen chose to avoid amniocentesis based on a negative cfDNA screen. All pregnancies had normal outcomes.</td></tr>
</table>

Citation Setting NCT Number	Test Results	Test Performance	Test Failures	Pregnancy and Other Outcomes
		Not reported **All trisomies (T21, T18, T13) cfDNA vs. reference standard** Sensitivity: 100% (95% CI, 54.1% to 100%) Specificity: 99.8% (95% CI, 99.4% to 99.98%) Condition prevalence: 0.5% (95% CI, 0.2% to 1.1%) PPV: 75% (95% CI, 42.9% to 92.3%) NPV: 100% Accuracy: 99.8% (95% CI, 99.4% to 99.98%) **All trisomies (T21, T18, T13) Standard screening vs. reference standard** PPV: 7.4% (95% CI, 2.4% to 16.0%) (2x2 table not reported)		If cfDNA was used as the only primary screen, up to 62 amniocenteses would have been avoided. Invasive procedure rate with cfDNA and standard screening: 2% (23 of 1,165: 95% CI, 1.3% to 3%) Estimated invasive procedure rate with standard screening and ultrasound: 6.8% (79 of 1,165: 95% CI, 5.4% to 8.4%) Invasive procedure rate after a negative cfDNA screen: 1.2% (14 of 1,151: 95% CI, 10.7% to 2%) One patient who had a negative traditional screen and a normal ultrasound was positive for T18 by cfDNA analysis and underwent amniocentesis that showed a normal chromosomal complement. 651 women underwent NT as part of screening: 6 (0.92%: 95% CI, 0.34% to 2%) had NT $\geq$ 3.5 mm; 3 of 6 the had a positive cfDNA screen for T21; the other 3 had a negative cfDNA test (3 of 640 with a negative cfDNA screen; 0.47%:

<table>
<tr><th>Citation
Setting
NCT Number</th><th>Test Results</th><th>Test Performance</th><th>Test Failures</th><th>Pregnancy and Other Outcomes</th></tr>
<tr><td></td><td></td><td></td><td></td><td>95% CI, 0.1% to 1.36%); 1 had a normal outcome; 1 had a spontaneous abortion with normal chromosomes; and 1 had an intrauterine fetal demise, with monosomy 21

Overall, 640 NT measurements were carried out to identify 2 nonviable pregnancies that would otherwise have been recognized clinically before 20 weeks' gestation.</td></tr>
<tr><td>Nicolaides et al., 2012[13]

U.K

Not reported</td><td>T21
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>8</td><td>0</td><td>0</td><td>1,941</td></tr></table>T18
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>2</td><td>2</td><td>0</td><td>1,945</td></tr></table>T21 and T18
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>10</td><td>2</td><td>0</td><td>1,937</td></tr></table>All trisomies (T21, T18, and T13)
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>10</td><td>2</td><td>0</td><td>1,937</td></tr></table></td><td>T21
cfDNA vs. reference standard
Sensitivity: 100% (95% CI, 63.1% to 100%)
Specificity: 100% (95% CI, 99.8% to 100%)

Condition prevalence: 0.4% (95% CI, 0.2% to 0.8%)

PPV: 100%
NPV: 100%

Accuracy: 100% (95% CI, 99.8% to 100%)

T21
Standard screening vs. reference standard
No information

T18
cfDNA vs. reference standard</td><td>Test failures: 100 of 2,049 (4.9%)

46 (2.2%) low fetal fraction
54 (2.6%) assay failure

One of the T18 cases failed to generate an assay result.</td><td>Birth outcomes: 2,038 (99.5%) euploid, 8 (0.4%) T21, 3 (0.1%) T18

Expected birth outcomes, based on maternal age distribution and age-related risk at 11 to 13 weeks: 7.89 T21, 3.21 T18</td></tr>
</table>

Citation Setting NCT Number	Test Results	Test Performance	Test Failures	Pregnancy and Other Outcomes
	See also Ashoor et al., 2013[9] for T13 results	Sensitivity: 100% (95% CI, 15.8% to 100%) Specificity: 99.9% (95% CI, 99.6% to 99.99%) Condition prevalence: 0.1% (95% CI, 0.01% to 0.4%) PPV: 50% (95% CI, 20.2% to 80.0%) NPV: 100% Accuracy: 99.9% (95% CI, 99.6% to 99.99%) **T18** **Standard screening vs. reference standard** No information **T21 and T18** **cfDNA vs. reference standard** Sensitivity: 100% (95% CI, 69.2% to 100%) Specificity: 99.9% (95% CI, 99.6% to 99.99%) Condition prevalence: 0.5% (95% CI, 0.2% to 0.9%) PPV: 83.3% (95% CI, 55.6% to 95.2%) NPV: 100% Accuracy: 99.9% (95% CI, 99.6% to 99.99%)		

Citation Setting NCT Number	Test Results	Test Performance	Test Failures	Pregnancy and Other Outcomes
		T21 and T18 **Standard screening vs. reference standard** FP rate with FTS: 87 of 1,939 (4.5%) FP rate with FTS + ultrasound: 59 of 1,939 (3.0%) **All trisomies (T21, T18, T13)** **cfDNA vs. reference standard** Sensitivity: 90.0% (95% CI, 68.3% to 98.8%) Specificity: 99.6% (95% CI, 99.6% to 99.97%) Condition prevalence: 0.5% (95% CI, 0.2% to 0.9%) PPV: 85.7% (95% CI, 65.7% to 94.9%) NPV: 99.9% (95% CI, 99.6% to 99.97%) Accuracy: 99.7% (95% CI, 99.4% to 99.92%) **Other measures** Median estimated risk for T21: 1:8,547 (range, 1:2 to 1:23,527) euploid, 1:2 (range, 1:2 to 1:3) T21, 1:6 (range, 1:4 to 1:13) T18 Both T21 and T18 risks were statistically significantly higher		

<table>
<tr><th>Citation
Setting
NCT Number</th><th>Test Results</th><th>Test Performance</th><th>Test Failures</th><th>Pregnancy and Other Outcomes</th></tr>
<tr><td></td><td></td><td>than the euploid group (P < .025)

Median estimated risk for T18: 1:14,980 (range, 1:3 to 1:47,472) euploid, 1:177 (range, 1:2 to 1:1,562) T21, 1:2 (range, not reported) T18;
both T21 and T18 risks were statistically significantly higher than the euploid group (P < .025)</td><td></td><td></td></tr>
<tr><td>Norton et al., 2015[14]

U.S., Belgium, Canada, Italy, Netherlands, and Sweden

NCT01511458</td><td>T21
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>38</td><td>9</td><td>0</td><td>15,794</td></tr></table>T21
Standard screening vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>30</td><td>854</td><td>8</td><td>14,949</td></tr></table>T21 and maternal age < 35 years
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>19</td><td>6</td><td>0</td><td>11,969</td></tr></table>T21 and low risk (< 1 in 270 on standard screening)
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>8</td><td>8</td><td>0</td><td>14,941</td></tr></table></td><td>T21
cfDNA vs. reference standard
Sensitivity: 100% (95% CI, 90.8% to 100%)
Specificity: 99.9% (95% CI, 99.9% to 99.97%)

Condition prevalence: 0.2% (95% CI, 0.2% to 0.3%)

PPV: 80.8% (95% CI, 68.7% to 89.0%)[a]
NPV: 100%

Accuracy: 99.9% (95% CI, 99.9% to 99.97%)

T21
Standard screening vs. reference standard
Sensitivity: 78.9% (95% CI, 62.7% to 90.5%)
Specificity: 94.6% (95% CI, 94.2% to 94.9%)</td><td>Test failures: 488 of 16,329 (3.0%) had no cfDNA result,

Of the 16,329 women, 192 (1.2%) had a fetal fraction < 4%, 83 (0.5%) had a fetal fraction that could not be measured, and 213 (1.3%) had a high assay variance or assay failure.

Median maternal weight: 93.7 kg in women with a low fetal fraction vs. 65.8 kg in women with a successful cfDNA test (P < .001)</td><td>Genetic testing: 625 of 15,841 (3.9%) any, 135 of 625 (21.6%) CVS, 422 of 625 (67.5%) amniocentesis, 16 of 625 (2.6%) products of conception, 52 of 625 (8.3%) newborn

Pregnancy outcome: 15,715 (99.2%) live birth, 62 (0.4%) termination, 17 (0.1%) stillbirth, 24 (0.2%) miscarriage, 23 (0.1%) birth outcome unknown but invasive prenatal test results available

Birth outcomes in women with no cfDNA results: 3 (0.6%) T21, (0.2%) T18, 2 (0.4%) T13, 4 (0.8%) triploidy, 1 (0.2%) T16 mosaic, 1 (0.2%) deletion 11p, 1 (0.2%) structurally abnormal chromosome</td></tr>
</table>

<table>
<tr><th>Citation
Setting
NCT Number</th><th>Test Results</th><th>Test Performance</th><th>Test Failures</th><th>Pregnancy and Other Outcomes</th></tr>
<tr>
<td></td>
<td>
T18
cfDNA vs. reference standard
<table>
<tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr>
<tr><td>9</td><td>1</td><td>1</td><td>15,830</td></tr>
</table>
T18
Standard screening vs. reference standard
<table>
<tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr>
<tr><td>8</td><td>49</td><td>2</td><td>15,782</td></tr>
</table>
T13
cfDNA vs. reference standard
<table>
<tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr>
<tr><td>2</td><td>2</td><td>0</td><td>11,181</td></tr>
</table>
T13
Standard screening vs. reference standard
<table>
<tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr>
<tr><td>1</td><td>28</td><td>1</td><td>11,155</td></tr>
</table>
</td>
<td>
Condition prevalence: 0.2% (95% CI, 0.2% to 0.3%)

PPV: 3.4% (95% CI, 2.9% to 4.0%)[a]
NPV: 99.9 % (95% CI, 99.9% to 99.97%)

Accuracy: 94.6% (95% CI, 94.2% to 94.9%)

T21 and maternal age < 35 years
cfDNA vs. reference standard
Sensitivity: 100% (95% CI, 82.4% to 100%)
Specificity: 99.95% (95% CI, 99.9% to 99.98%)

Condition prevalence: 0.2% (95% CI, 0.1% to 0.2%)

PPV: 76.0% (95% CI, 58.7% to 87.6%)[a]
NPV: 100%

Accuracy: 99.95% (95% CI, 99.89% to 99.98%)

T21 and low risk (< 1 in 270 on standard screening)
cfDNA vs. reference standard
Sensitivity: 100% (95% CI, 63.0% to 100%)
</td>
<td></td>
<td>
Prevalence of aneuploidies: 1 in 236 (0.4%) in women with a successful cfDNA test vs. 1 in 38 (2.7%) in women with a failed cfDNA test (P < .001)

Prevalence of aneuploidies in women with a fetal fraction < 4%: 9 of 192 (4.7%)

Standard screening detected the 6 common aneuploidies where there was no cfDNA result, with risks ranging from 1 in 26 to 1 in 2
</td>
</tr>
</table>

Citation Setting NCT Number	Test Results	Test Performance	Test Failures	Pregnancy and Other Outcomes
		Specificity: 99.95% (95% CI, 99.89% to 99.98%) Condition prevalence: 0.05% (95% CI, 0.02% to 0.1%) PPV: 50.0% (95% CI, 33.3% to 66.7%)[a] NPV: 100% Accuracy: 99.95% (95% CI, 99.89% to 99.98%) **T18** **cfDNA vs. reference standard** Sensitivity: 90.0% (95% CI, 55.5% to 99.8%) Specificity: 99.99% (95% CI, 99.96% to 100%) Condition prevalence: 0.06% (95% CI, 0.03% to 0.1%) PPV: 90.0% (95% CI, 55.6% to 98.5%)[a] NPV: 99.99% (95% CI, 99.96% to 100%) Accuracy: 99.99% (95% CI, 99.95% to 100%) **T18** **Standard screening vs. reference standard** Sensitivity: 80.0% (95% CI, 44.4% to 97.5%)		

Citation Setting NCT Number	Test Results	Test Performance	Test Failures	Pregnancy and Other Outcomes
		Specificity: 99.69% (95% CI, 99.59% to 99.77%) Condition prevalence: 0.06% (95% CI, 0.03% to 0.7%) PPV: 14.0% (95% CI, 9.7% to 19.9%)[a] NPV: 99.99% (95% CI, 99.96% to 100%) Accuracy: 99.68% (95% CI, 99.58% to 99.76%) **T13** **cfDNA vs. reference standard** Sensitivity: 100% (95% CI, 15.8% to 100%) Specificity: 99.98% (95% CI, 99.94% to 100%) Condition prevalence: 0.02% (95% CI, 0% to 0.06%) PPV: 50.0% (95% CI, 20.0% to 80.0%)[a] NPV: 100% Accuracy: 99.98% (95% CI, 99.94% to 100%) **T13** **Standard screening vs. reference standard** Sensitivity: 50.0% (95% CI, 1.3% to 98.7%)		

<table>
<tr><th>Citation
Setting
NCT Number</th><th>Test Results</th><th>Test Performance</th><th>Test Failures</th><th>Pregnancy and Other Outcomes</th></tr>
<tr><td></td><td></td><td>Specificity: 99.75% (95% CI, 99.64% to 99.83%)

Condition prevalence: 0.02% (95% CI, 0% to 0.06%)

PPV: 3.4% (95% CI, 0.8% to 13.0%)[a]
NPV: 99.95 % (95% CI, 99.96% to 100%)

Accuracy: 99.7% (95% CI, 99.6% to 99.8%)

Other measures
Median NT: 0.98 MoM (SD $\log_{10}$, 0.09)</td><td></td><td></td></tr>
<tr><td>Pergament et al., 2014[16]

U.S., Czech Republic, Japan, Turkey, Ireland, Spain, and Poland

Not reported</td><td>T21
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>1</td><td>0</td><td>0</td><td>473</td></tr></table>T18
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>2</td><td>0</td><td>0</td><td>472</td></tr></table>T13
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>0</td><td>0</td><td>0</td><td>474</td></tr></table>45,X
cfDNA vs. reference standard</td><td>T21
cfDNA vs. reference standard
Sensitivity: 100% (95% CI, 2.5% to 100%)
Specificity: 100% (95% CI, 99.2% to 100%)

Condition prevalence: 0.2% (95% CI, 0.01% to 1.2%)

PPV: 100%
NPV: 100%

Accuracy: 100% (95% CI, 99.2% to 100%)

T18
cfDNA vs. reference standard</td><td>Overall, 85 tests were ‘no-calls’ (8.1%) in both risk groups.

‘No-call’ rate: 8.5% low-risk women; 8.1% high-risk women; P = 0.86); this was attributed to the lower gestational age in the low-risk cohort compared with the overall cohort (12.9 weeks vs. 14.3 weeks)

P value distributions for chromosomes 13, 18, and 21 in each cohort were compared and no significant differences were found for any of the 3 between the low- and high-risk cohorts.</td><td>Not reported</td></tr>
</table>

Citation Setting NCT Number	Test Results	Test Performance	Test Failures	Pregnancy and Other Outcomes
	TP | FP | FN | TN 2 | 0 | 0 | 472 **All trisomies (T21, T18, T13) cfDNA vs. reference standard** TP | FP | FN | TN 3 | 0 | 0 | 471 **All trisomies (T21, T18, T13) and 45,X cfDNA vs. reference standard in low-risk women** TP | FP | FN | TN 5 | 0 | 0 | 469 **cfDNA vs. reference standard in high-risk women** TP | FP | FN | TN 98 | 2 | 2 | 389	Sensitivity: 100% (95% CI, 15.8% to 100%) Specificity: 100% (95% CI, 99.2% to 100%) Condition prevalence: 0.4% (95% CI, 0.05% to 1.5%) PPV: 100% NPV: 100% Accuracy: 100% (95% CI, 99.2% to 100%) **T13** **cfDNA vs. reference standard** Sensitivity: 100% (95% CI, 99.2% to 100%) Specificity: Not calculable Condition prevalence: 0% (95% CI, 0% to 0.8%) PPV: Not calculable NPV: 100% Accuracy: 100% (95% CI, 99.2% to 100%) **45,X** **cfDNA vs. reference standard** Sensitivity: 100% (95% CI, 15.8% to 100%) Specificity: 100% (95% CI, 99.2% to 100%)		

Citation Setting NCT Number	Test Results	Test Performance	Test Failures	Pregnancy and Other Outcomes
		Condition prevalence: 0.4% (95% CI, 0.05% to 1.5%) PPV: 100% NPV: 100% Accuracy: 100% (95% CI, 99.2% to 100%) **All trisomies (T21, T18, T13) cfDNA vs. reference standard** Sensitivity: 100% (95% CI, 29.2% to 100%) Specificity: 100% (95% CI, 99.2% to 100%) Condition prevalence: 0.6% (95% CI, 0.1% to 1.8%) PPV: 100% NPV: 100% Accuracy: 100% (95% CI, 99.2% to 100%) **All trisomies (T21, T18, T13) and 45,X cfDNA vs. reference standard low-risk women** Sensitivity: 100% (95% CI, 47.8% to 100%) Specificity: 100% (95% CI, 99.2% to 100%) Condition prevalence: 1.0% (95% CI, 0.3% to 2.4%)		

<table>
<tr><th>Citation
Setting
NCT Number</th><th>Test Results</th><th>Test Performance</th><th>Test Failures</th><th>Pregnancy and Other Outcomes</th></tr>
<tr><td></td><td></td><td>PPV: 100%
NPV: 100%

Accuracy: 100% (95% CI, 99.2% to 100%)

High-risk women
Sensitivity: 98.0% (95% CI, 93.0% to 99.7%)
Specificity: 99.5% (95% CI, 98.2% to 99.9%)

Condition prevalence: 20.4% (95% CI, 16.9% to 24.2%)

PPV: 98.0% (95% CI, 92.5% to 99.5%)
NPV: 99.5% (95% CI, 98.0% to 99.9%)

Accuracy: 99.2% (95% CI, 97.9% to 99.8%)</td><td></td><td></td></tr>
<tr><td>Quezada et al., 2015[17]

U.K.

Not reported</td><td>T21
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>32</td><td>1</td><td>0</td><td>2,752</td></tr></table>T18
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>9</td><td>5</td><td>1</td><td>2,770</td></tr></table>T13
cfDNA vs. reference standard</td><td>T21
cfDNA vs. reference standard
Sensitivity: 100% (95% CI, 89.1% to 100%)
Specificity: 99.96% (95% CI, 99.80% to 100%)

Condition prevalence: 1.2% (95% CI, 0.8% to 1.6%)

PPV: 97.0% (95% CI, 81.9% to 99.6%)
NPV: 100%</td><td>Initial cfDNA failure: 123 of 2,905 (4.2%)

Subsequent cfDNA failure: 41 of 110 (37.3%)

No cfDNA results: 54 of 2,905 (1.9%) (1 sample not received at the lab, 38 with fetal fraction < 4%, 15 assay failures)</td><td>Birth outcomes in the 54 cases with no cfDNA result: 49 with no T21, T18, or T13; 2 with T21; 0 with T18; 0 with T13; 3 miscarriages with no karyotype</td></tr>
</table>

Citation Setting NCT Number	Test Results	Test Performance	Test Failures	Pregnancy and Other Outcomes
	TP | FP | FN | TN 2 | 2 | 3 | 2,778 **All trisomies (T21, T18, T13) cfDNA vs. reference standard** TP | FP | FN | TN 43 | 8 | 4 | 2,730 **All trisomies (T21, T18, T13) Standard screening vs. reference standard** TP | FP | FN | TN 49 | 124 | 0 | 2,663	Accuracy: 99.96% (95% CI, 99.80% to 100%) **T21** **Standard screening vs. reference standard** No information **T18** **cfDNA vs. reference standard** Sensitivity: 90.0% (95% CI, 55.5% to 99.8%) Specificity: 99.8% (95% CI, 99.6% to 99.9%) Condition prevalence: 0.4% (95% CI, 0.2% to 0.7%) PPV: 64.3% (95% CI, 42.3% to 81.6%) NPV: 99.96% (95% CI, 99.77% to 99.99%) Accuracy: 99.78% (95% CI, 99.53% to 99.92%) **T18** **Standard screening vs. reference standard** No information **T13** **cfDNA vs. reference standard** Sensitivity: 40.0% (95% CI, 5.3% to 85.3%)		

Citation Setting NCT Number	Test Results	Test Performance	Test Failures	Pregnancy and Other Outcomes
		Specificity: 99.9% (95% CI, 99.7% to 99.99%) Condition prevalence: 0.2% (95% CI, 0.06% to 0.4%) PPV: 50.0% (95% CI, 14.7% to 85.2%) NPV: 99.9% (95% CI, 99.8% to 99.95%) Accuracy: 99.8% (95% CI, 99.6% to 99.9%) **T13** **Standard screening vs. reference standard** No information **All trisomies (T21, T18, T13)** **cfDNA vs. reference standard** Sensitivity: 91.5% (95% CI, 79.6% to 97.6%) Specificity: 99.7% (95% CI, 99.4% to 99.9%) Condition prevalence: 1.7% (95% CI, 1.2% to 2.2%) PPV: 84.3% (95% CI, 72.8% to 91.5%) NPV: 99.8% (95% CI, 99.6% to 99.8%) Accuracy: 99.6% (95% CI, 99.3% to 99.8%)		

<table>
<tr><th>Citation
Setting
NCT Number</th><th>Test Results</th><th>Test Performance</th><th>Test Failures</th><th>Pregnancy and Other Outcomes</th></tr>
<tr><td></td><td></td><td>All trisomies (T21, T18, T13)
Standard screening vs. reference standard
Sensitivity: 100% (95% CI, 92.7% to 100%)
Specificity: 95.6% (95% CI, 94.7% to 96.3%)

Condition prevalence: 1.7% (95% CI, 1.3% to 2.3%)

PPV: 28.3% (95% CI, 25.0% to 31.9%)
NPV: 100%

Accuracy: 95.6% (95% CI, 94.8% to 96.4%)</td><td></td><td></td></tr>
<tr><td colspan="5">Twin Pregnancies</td></tr>
<tr><td>del Mar Gil et al., 2014[11]

U.K.

Not reported</td><td>T21
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>9</td><td>0</td><td>1</td><td>182</td></tr></table>T18
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>0</td><td>0</td><td>0</td><td>192</td></tr></table>T13
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>1</td><td>0</td><td>0</td><td>191</td></tr></table>All trisomies (T21, T18, T13)</td><td>T21
cfDNA vs. reference standard
Sensitivity: 90.0% (95% CI, 55.5% to 99.8%)
Specificity: 100% (95% CI, 98.0% to 100%)

Condition prevalence: 5.2% (95% CI, 2.5% to 9.4%)

PPV: 100%
NPV: 99.4% (95% CI, 96.6% to 99.9%)

Accuracy: 99.5% (95% CI, 97.1% to 99.99%)</td><td>15 (7.2%) no cfDNA results (11 low fetal fraction, 4 laboratory processing issues)</td><td>Not reported</td></tr>
</table>

<table>
<tr><th>Citation
Setting
NCT Number</th><th>Test Results</th><th>Test Performance</th><th>Test Failures</th><th>Pregnancy and Other Outcomes</th></tr>
<tr><td></td><td>cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>10</td><td>0</td><td>1</td><td>181</td></tr></table></td><td>T18
cfDNA vs. reference standard
Sensitivity: Not calculable
Specificity: 100% (95% CI, 98.1% to 100%)

Condition prevalence: 0% (95% CI, 0% to 1.9%)

PPV: Not calculable %
NPV: 100%

Accuracy: 100% (95% CI, 98.1% to 100%)

T13
cfDNA vs. reference standard
Sensitivity: 100% (95% CI, 2.5% to 100%)
Specificity: 100% (95% CI, 98.1% to 100%)

Condition prevalence: 0.5% (95% CI, 0.01% to 2.9%)

PPV: 100%
NPV: 100%

Accuracy: 100% (95% CI, 98.1% to 100%)

All trisomies (T21, T18, and T13)
cfDNA vs. reference standard
Sensitivity: 90.9% (95% CI, 58.7% to 99.8%)</td><td></td><td></td></tr>
</table>

<table>
<tr><th>Citation
Setting
NCT Number</th><th>Test Results</th><th>Test Performance</th><th>Test Failures</th><th>Pregnancy and Other Outcomes</th></tr>
<tr><td></td><td></td><td>Specificity: 100% (95% CI, 98.0% to 100%)

Condition prevalence: 5.7% (95% CI, 2.9% to 10.0%)

PPV: 100%
NPV: 99.4% (95% CI, 96.5% to 99.9%)

Accuracy: 99.5% (95% CI, 96.5% to 99.9%)</td><td></td><td></td></tr>
<tr><td colspan="5">Mixed Singleton and Twin Pregnancies</td></tr>
<tr><td>Palomaki et al., 2017[15,92]

U.S.

NCT01966991</td><td>T21
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>7</td><td>2</td><td>0</td><td>2,522</td></tr></table>T18
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>3</td><td>0</td><td>0</td><td>2,528</td></tr></table>T13
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>2</td><td>2</td><td>0</td><td>2,527</td></tr></table>All trisomies (T21, T18, T13)
cfDNA vs. reference standard<table><tr><th>TP</th><th>FP</th><th>FN</th><th>TN</th></tr><tr><td>12</td><td>4</td><td>0</td><td>2,515</td></tr></table></td><td>T21
cfDNA vs. reference standard
Sensitivity: 100% (95% CI, 59.0% to 100%)
Specificity: 99.92% (95% CI, 99.7% to 99.9%)

Condition prevalence: 0.3% (95% CI, 0.1% to 0.6%)

PPV: 77.8% (95% CI, 46.7% to 93.3%)
NPV: 100%

Accuracy: 99.9% (95% CI, 99.7% to 99.99%)

T18
cfDNA vs. reference standard
Sensitivity: 100% (95% CI, 29.2% to 100%)</td><td>Initial cfDNA failure rate: 2.6% (150 of 2,681; 95% CI, 4.8% to 6.5%)

Subsequent negative cfDNA screen: 65 (43%)
Negative serum screen: 54 (36%)
Declined further testing: 13 (9%)
Positive serum screen: 9 (6%)
Pregnancy loss: 4 (3%)
Unknown: 5 (3%)

Of the 9 serum-positive screen pregnancies, 8 had normal birth outcomes. The ninth woman was diagnosed with a mosaic condition after a positive cfDNA test from another sequencing laboratory; a normal female infant was delivered.</td><td>Of the 16 women with positive cfDNA screens, 12 were TP and 4 were FP. All were confirmed by invasive testing and diagnostic testing. Nine TPs were confirmed and 7 (78%) were terminated. There were 2 cases of monozygotic twins with screen-negative cfDNA results (SNP-based tests do not identify monozygotic twins).</td></tr>
</table>

Citation Setting NCT Number	Test Results	Test Performance	Test Failures	Pregnancy and Other Outcomes
		Specificity: 100% (95% CI, 99.8% to 100%) Condition prevalence: 0.1% (95% CI, 0.02% to 0.4%) PPV: 100% NPV: 100% Accuracy: 100% (95% CI, 99.9% to 100%) **T13** **cfDNA vs. reference standard** Sensitivity: 100% (95% CI, 15.8% to 100%) Specificity: 99.9% (95% CI, 99.7% to 99.99%) Condition prevalence: 0.1% (95% CI, 0.01% to 0.3%) PPV: 50.0% (95% CI, 20.0% to 80.0%) NPV: 100% Accuracy: 99.9% (95% CI, 99.7% to 99.99%) **All trisomies (T21, T18, T13)** **cfDNA vs. reference standard** Sensitivity: 100% (95% CI, 73.5% to 100%) Specificity: 99.8% (95% CI, 99.6% to 99.96%)	None of 150 women chose invasive testing. 13 additional sex chromosome failures occurred (0.5%) DNA failures were strongly associated with maternal weight of 80 kg or higher (RR, 11.4; 95% CI, 6.3 to 21; *P* < .001).	

Citation Setting NCT Number	Test Results	Test Performance	Test Failures	Pregnancy and Other Outcomes
		Condition prevalence: 0.5% (95% CI, 0.2% to 0.8%) PPV: 75.0% (95% CI, 53.0% to 88.9%)[a] NPV: 100% Accuracy: 99.8% (95% CI, 99.6% to 99.96%) **45,X** Three of 2,681 (0.11%; 95% CI, 0.03% to 0.3%)) were screen-positive. Of these, 2 were true positives and ended in spontaneous losses. The third resulted in a late-first-trimester fetal loss with no diagnostic information. **Sex trisomy** Optional sex trisomy (and fetal sex) interpretations were chosen by 91.2% of the women (2,445 of 2,681). Of these, 2 were screen-positive for a sex trisomy; both women received posttest genetic counseling, and both declined prenatal diagnostic testing. Both infants were live born; one was confirmed by postnatal karyotype.		

Abbreviations. 45,X: Turner syndrome; AFP: alpha-fetoprotein; cfDNA: cell-free DNA; CI: confidence interval; CVS: chorionic villus sampling; FN: false negative; FP: false positive; FTS: first-trimester screening; hCG: human chorionic gonadotrophin; IQR: interquartile range; MoM: multiples of the median; NPV: negative predictive value; NT: nuchal translucency; PAPP-A: pregnancy-associated plasma protein A; PPV: positive predictive value; RR: risk ratio; SCA: sex chromosome abnormality; SD: standard deviation; SIPS:

first-trimester PAPP-A, second-trimester AFP, hCG, uE3, inhibin A; T13: trisomy 13; T16: trisomy 16; T18: trisomy 18; T21: trisomy 21; TN: true negative; TP: true positive; uE3: unconjugated estriol 3. Note. We used MedCalc (https://www.medcalc.org/calc/diagnostic_test.php) to calculate a standard set of test performance measures, which may not have been reported in the published paper. [a] Results from MedCalc were different to those reported in the published paper.

Table C7. Study Characteristics and Evidence Tables for Economic Studies

Citation Setting NCT Number	Design Test Comparator(s)	Population Analytic Assumptions	Main Findings
Benn et al., 2015[18] U.S. Not reported	Aim: To analyze the economic value of replacing conventional fetal aneuploidy screening approaches with cfDNA in the general pregnancy population Design: Economic analysis, using a decision-analytic model Test: cfDNA screening (test not named) Comparator: Conventional, combined first-trimester screening comprising NT together with maternal serum markers, PAPP-A, and free hCG at 12 weeks' gestational age), in combination with second-trimester screening tests as appropriate	Population: Theoretical cohort of 3,952,841 live births, representing the U.S. general obstetric prenatal screening population in 2012 Conditions: T21, T18, T13, and 45,X Analytic assumptions: • Perspective not defined • Time horizon not defined (lifetime costs for conditions) • Costs in 2014 U.S. dollars • Costs from CMS Fee Schedules increased by 20% to reflect private or commercial payer costs • Annual discount rate not defined • Conventional screening rate = 100% (range, 0% to 100%) • cfDNA screening rate = 66% (range, 0% to 100%) • Invasive testing rate for conventional screening FPs = 45% (range, 25% to 100%) • Termination rates = 65% to 90% by condition (range, 0% to 100%) • Cost of first-trimester screening = $369 (range, $222 to $443) • Cost of sequential screening = $136 (range, $82 to $164	Cost-per-case, if cfDNA screening were cost neutral: • All pregnant women in the first trimester = $744 • Women of AMA (≥ 35 years) = $1,474 • All pregnant women in the second trimester = $486 Impact: • Conventional screening with cfDNA would increase the number of affected pregnancies prenatally detected by 12.4% (1403 of 11,314) in the general screening population. • Replacing conventional screening with cfDNA would reduce the number of affected births by 33.4% (1,213 of 3,629) in the general screening population. • cfDNA is associated with a 60.0% (36,834 of 61,430) reduction in the number of invasive tests performed in the general screening population. • As a result, the number of procedure-related euploid fetal losses is also reduced with cfDNA, with a 73.5% (194 of 264) reduction in the general screening population. See Table C9 for other pregnancy outcomes

Citation Setting NCT Number	Design Test Comparator(s)	Population Analytic Assumptions	Main Findings
		See Table C8 for incidence rates and test performance assumptions Cost of invasive testing (amniocentesis/CVS) = $835/$892 (range, $501 to $1,070) Lifetime costs of T21 = $677,000 (range, $541,600 to $812,400) Lifetime costs of T18 = $29,307 (range, $23,446 to $35,168) Lifetime costs of T13 = $33,577 (range, $26,862 to $40,292) Lifetime costs of 45,X = $271,010 (range, $216,808 to $325,212)	
Crimmins et al., 2017[19] U.S. Not reported	Aim: To compare the unit cost of noninvasive prenatal testing (cfDNA) in an urban population that did not have FTS as a primary screening tool for T21 to multiple marker screening (QUAD) Design: Cost-sensitivity analysis using a decision-analytic model Test: cfDNA screening (test not named) Comparator:	Population: 590 pregnant women choosing aneuploidy risk assessment who presented for care between 15 and 21 weeks at a single urban center Condition: T21 Analytic assumptions: • Perspective not defined • Time horizon not defined • Costs in U.S. dollars, year of costs not defined • Annual discount rate not defined • cfDNA FP rate = 0.06% • cfDNA FN rate = 0% • All participants who screened positive proceeded with amniocentesis	Cost sensitivity: cfDNA and QUAD screen would be cost equivalent at $360.66 Impact: If cfDNA were used as the primary method of second-trimester screening, regardless of *a priori* risk: • Rate of invasive procedures would be reduced by 55.4% and rate of procedure-related losses would be reduced from 65 to 28 (57% reduction) • Number of women having genetic counseling would be reduced by 78% (14.7% versus 2.9% of the population)

Citation Setting NCT Number	Design Test Comparator(s)	Population Analytic Assumptions	Main Findings
	QUAD screening in the second trimester comprising MSS between 15 and 22 weeks and a level II anatomy ultrasound between 18 and 22 weeks	• Rate of uninformative cfDNA tests = 2.58% • Procedure-related loss from amniocentesis = 1 in 1,000 • Cost of QUAD screen = $419.00 • Cost of cfDNA = $0 to $3,000 (sensitivity analysis) • Cost of AFP = $99.00 • Cost of genetic counselling (30 mins) = $160.00 • Cost of amniocentesis = $1,100.00 • Cost of procedure-related loss = $1,649.00 • Cost of elective termination = $1,649.00 • Local charge of QUAD screen for Medicaid clients = $415 • Local charge of maternal serum AFP screen for Medicaid clients = $99	
Evans et al., 2015[20] U.S. Not reported	Aim: To determine whether implementation of primary cfDNA screening would be cost-effective and to evaluate potential lower-cost alternatives Design: Economic analysis using a decision-analytic model Test: cfDNA (test not named) screening	Population: Theoretical cohort of 1,000,000 pregnant women (no further details reported, but described as at low risk) Condition: T21 Analytic assumptions: • Public policy perspective • Time horizon not defined (lifetime costs included for each T21 case) • Costs in U.S. dollars, year of costs not defined • Annual discount rate not defined	Cost per patient: See Table C10 for costs per patient screened. • Cost of cfDNA per patient screened was $1,017 in the base case analysis • Marginal cost per viable case detected for the primary cfDNA screening strategy as compared to other strategies was greater than the cost of care for a missed case See Table C11 for costs per patient screened and marginal costs in the best-case scenario. Impact: See Table C12 for the expected numbers of patients tested.

Citation Setting NCT Number	Design Test Comparator(s)	Population Analytic Assumptions	Main Findings
	Comparators: Contingent: cfDNA for women at high risk after conventional FTS Hybrid: cfDNA for all women ≥ 35 years and women < 35 at high risk after FTS	• cfDNA screening acceptance rate = 100% (alternative value of 70%) • Invasive testing acceptance rate = 100% (alternative value of 70%) • Termination rate for affected fetuses = 100% (alternative value of 70%) • NT in addition to cfDNA screening = 0% (alternative value of 100%) • Detection rate (sensitivity) = 99.3% (alternative of 99.9%) • FP rate = 0.16% (alternative of 0.1%) • Cost of biochemical screening = $41 (alternative of $64) • Cost of NT examination = $123 (alternative of $147) • Cost of counseling screen-positive women = $73 (alternative of $88) • Cost of counseling cfDNA-positive women = $144 (alternative of $173) • Cost of CVS and karyotyping = $775 (alternative of $930) • Cost of amniocentesis and karyotyping = $687 (alternative of $825) • Cost of cfDNA screening = $1,000 (alternative of $700) • Cost of care per missed case = $1,055,925	
Fairbrother et al., 2016[21] U.S.	Aim: To compare the cost effectiveness of prenatal screening for common fetal trisomies with FTS or cfDNA screening within a	Population: Theoretical cohort of 4,000,000 pregnant women representative of the U.S. general obstetric prenatal screening population in 1 year	Cost analysis: • Total costs of FTS screening = $3.88 billion • Cost per case identified through FTS = $497,909 • At a cost of $453 or less, cfDNA screening was cost saving compared with FTS.

Citation Setting NCT Number	Design Test Comparator(s)	Population Analytic Assumptions	Main Findings
Not reported	representative general pregnancy population in the U.S. Design: Economic analysis using a decision-analytic model Test: cfDNA screening (test not named) Comparator: FTS, comprising ß-hCG, PAPP-A, and NT	Conditions: T21, T18, and T13 Analytic assumptions: • Perspective not defined • Time horizon not defined (although costs appear to include lifetime costs for conditions) • Costs in 2014 U.S. dollars • Annual discount rate not defined • Screening uptake rate for FTS = 70% • Screening uptake rate for cfDNA screening = 70% • T21 prevalence = 1 in 530 (range, 1 in 450 to 1 in 600) • T18 prevalence = 1 in 1,100 (range, 1 in 900 to 1 in 1,500) • T13 prevalence = 1 in 3,500 (range, 1 in 2,500 to 1 in 5,000) • FTS cumulative FP rate = 5% (range, 3% to 7%) • FTS sensitivity, T21 = 85% (range 75% to 90%) • FTS sensitivity, T18 = 84% (range 80% to 90%) • FTS sensitivity, T13 = 84% (range 80% to 90%) • Patients referred out for screening = 35% (25% to 50%) • cfDNA screening cumulative FP rate = 0.3% (range, 0.1% to 0.5%) • cfDNA screening sensitivity, T21 = 99.0% (range 98.0% to 99.9%) • cfDNA screening sensitivity, T18 = 96.8% (range 90.0% to 99.9%)	• At a cost of $665, costs per case by cfDNA screening and FTS were equivalent. • In one-way sensitivity analysis, cfDNA screening remained the dominant strategy over FTS in all analyses, except when the cost of cfDNA screening exceeded $453. • At increased screening adherence with cfDNA screening of 75%, 80%, and 85%, cfDNA screening remained cost saving over FTS at a cfDNA screening unit cost up to $490, $522, and $550, respectively. Impact: • Number undergoing either FTS or cfDNA screening = 2.8 million out of the 4 million cohort • Number of trisomy cases identified by cfDNA screening = 8,993, of which 5,544 were T21, 2,710 were T18, and 738 were T13 • Number of trisomy cases identified by FTS = 7,799, of which 4,768 were T21, 2,356 were T18, and 674 were T13 • Number of invasive procedures with cfDNA screening = 17,303, of which 8,342 were unnecessary because of FP cfDNA screening results and 42 were normal fetal losses • Number of invasive procedures with FTS = 147,311, of which 139,540 were unnecessary because of FP FTS results and 698 were normal fetal losses • Compared to FTS, cfDNA screening identified 15% more trisomy cases, reduced invasive procedures by 88%, and reduced iatrogenic normal fetal loss by 94%.

Citation Setting NCT Number	Design Test Comparator(s)	Population Analytic Assumptions	Main Findings
		• cfDNA screening sensitivity, T13 = 92.1% (range 85.0% to 95.0%) • Termination rate, T21 = 75% (60% to 90%) • Termination rate, T18 = 90% (80% to 95%) • Termination rate, T13 = 90% (80% to 95%) • Procedure-related miscarriage = 0.5% (range, 0.2% to 2%) • Cost of cfDNA screening = $400 to $700 • Cost of first-trimester serum = $48.30 (range, $30 to $100) • Cost of NT = $122.51 (range, $100 to $300) • Cost of invasive procedure = $1,300 (range, $500 to $2,500) • Cost of office visit with counseling = $120 (range, $80 to $200) • Cost of T21 birth = $850,000 (range, $600,000 to $1,000,000) • Cost of T21 birth = $50,000 (range, $30,000 to $70,000) • Cost of T21 birth = $38,000 (range, $25,000 to $50,000) • Cost of termination = $600 (range, $400 to $1,000)	
Kaimal et al., 2015[22] U.S. Not reported	Aim: To use a decision-analytic model to assess a comprehensive set of outcomes of prenatal genetic testing strategies among women of varying ages	Population: Theoretical cohort of pregnant women desiring prenatal testing (screening or diagnostic or both) Conditions: T21, T18, T13, sex chromosome aneuploidy (45,X; 47,XXX; 47,XXY; 47,XYY), a pathogenic	Incremental cost-effectiveness ratio of cfDNA testing: See Table C14 for QALYs and costs. In probabilistic sensitivity analysis, when compared with cfDNA alone, multiple marker screening is dominant (more effective and less costly) 93.7% of the time and dominant or cost-effective at a $100,000 per QALY threshold. Impact:

Citation Setting NCT Number	Design Test Comparator(s)	Population Analytic Assumptions	Main Findings
	Design: Cost-effectiveness analysis using a decision-analytic model Test: cfDNA screening (test not named) Comparators: • Combined first- and second-trimester serum analytes and NT measurement in which women had only the option of diagnostic testing if additional information was desired after screening • Marker screening with the option of secondary cfDNA or diagnostic testing in which women could opt for either cfDNA screening or diagnostic testing if additional information was desired after initial screening • cfDNA screening with concurrent NT assessment, with diagnostic testing if	copy number variant (microdeletion or duplication) or other rare chromosomal abnormality, or a variant of uncertain significance Analytic assumptions: • Perspective not defined • Time horizon of a woman's lifetime for long-term outcomes with testing utilities applying for 12 months • Costs in U.S. dollars, year not reported • Annual discount rate of 3% • Maternal age = 30 years (range, 20 to 40) • Cost of serum screen = $338 • Cost of NT ultrasound = $222 • Cost of amniocentesis with CMA = $2,384 • Cost of cfDNA = $1,796 • Cost of termination or miscarriage = $938 • Cost of delivery = $8,445 See Table C13 for other model assumptions. Time tradeoff utilities were obtained from a diverse group of 281 women presenting for care at the University of California, San Francisco, prenatal care clinic or prenatal diagnosis center, or the San Francisco General Hospital prenatal care clinic.	See Table C15 for pregnancy outcomes.

Citation Setting NCT Number	Design Test Comparator(s)	Population Analytic Assumptions	Main Findings
	additional information was desired • Concurrent marker and cfDNA screening with diagnostic testing if additional information was desired • Diagnostic testing without prior screening		
Shiv et al., 2017[23] U.S. Not reported	Aim: To investigate potential costs and test performance of screening algorithms when accounting for detectable aneuploidies Design: Economic analysis (the model approach is not reported in detail) Test: cfDNA screening (test not named) Comparator: Sequential cfDNA screening	Population: Theoretical cohort of 3,000 pregnant women (no further details reported) Conditions: T21, all detectable aneuploidies (assumed to be T21, T18, T13, and sex chromosome aneuploidies) Analytic assumptions: • Perspective not defined • Time horizon not defined • Costs in U.S. dollars, year of costs not defined • Annual discount rate not defined • Screening rate = 74.5% • Rate of all aneuploidies = 0.39% • Rate of T21 = 0.23% • cfDNA screening detection rate (sensitivity), T21 = 99% • cfDNA screening FP rate, T21 = 0.5% • cfDNA screening detection rate (sensitivity), all aneuploidies = 70% • cfDNA screening FP rate, all aneuploidies = 1.5%	Cost analysis: Based on a cohort of 2,235 women screened (74.5% of 3,000 to reflect the institutional screening rate) See Table C16 for the costs of screening Impact: Down Syndrome • Universal cfDNA screening: positive tests, 16 (0.72%); FP tests, 11 (0.49%); invasive tests avoided, 101; additional aneuploidy cases detected, 1; aneuploidy cases missed, 0.05 • Sequential: positive tests, 117 (5.2%); FP tests, 112 (5%); invasive tests avoided, 0; additional aneuploidy cases detected, 0; aneuploidy cases missed, 0.25 All Aneuploidies Detectable • Universal cfDNA screening: positive tests, 40 (1.8%); FP tests, 34 (1.5%); invasive tests avoided, 59; additional aneuploidy cases detected, 0; aneuploidy cases missed, 3 • Sequential: positive tests, 99 (4.4%); FP tests, 92 (4.1%); invasive tests avoided, 0; additional aneuploidy cases detected, 1; aneuploidy cases missed, 2

<table>
<tr><th>Citation
Setting
NCT Number</th><th>Design
Test
Comparator(s)</th><th>Population
Analytic Assumptions</th><th>Main Findings</th></tr>
<tr><td></td><td></td><td>• Sequential screening detection rate (sensitivity), T21 = 95%
• Sequential screening FP rate, T21 = 5%
• Sequential screening detection rate (sensitivity), all aneuploidies = 81.6%
• Sequential screening FP rate, all aneuploidies = 4.11%</td><td></td></tr>
<tr><td>Walker et al., 2015[24]

U.S.

Not reported</td><td>Aim:
To determine the cost effectiveness of cfDNA as a replacement for integrated screening

Design:
Cost-effectiveness analysis using Monte-Carlo simulation

Test:
cfDNA screening (test not named)

Comparator:
Integrated MSS</td><td>Population:
Theoretical cohort of 1,000,000 pregnant women at 10 weeks of pregnancy

Conditions:
T21

Analytic assumptions:
• Societal perspective with stratified costs to reflect other perspectives
• Time horizon included lifetime costs associated with Down syndrome
• Costs in 2013 U.S. dollars
• Annual discount rate of 3%
• cfDNA detection rate = 99.5% (range, 98.6% to 99.9%)
• cfDNA FP rate = 0.12% (range, 0.08% to 0.17%)

See Table C17 for other analytic assumptions</td><td>Cost effectiveness:
• cfDNA screening dominates integrated screening (i.e., is more effective and cheaper), with an ICER of $-277,955 (95% CI, $-881,882 to $532,785) per case detected.
• cfDNA screening no longer dominated integrated screening when the unit cost of cfDNA exceeded $549, the lifetime costs of Down Syndrome were below $827,157 and cfDNA screening uptake was less than 69.5%.
• cfDNA screening was more effective than integrated screening 99.9% of the time and less costly than integrated screening 81.8% of the time.

<table><tr><th>Baseline Analysis Costs for 1,000,000 Women</th><th>cfDNA Screening</th><th>Integrated Screening</th></tr><tr><td>Screening</td><td>$324,298,422</td><td>$160,544,211</td></tr><tr><td>Diagnostic testing</td><td>$3,053,516</td><td>$14,411,432</td></tr><tr><td>Termination</td><td>$796,064</td><td>$1,294,473</td></tr><tr><td>Lifetime medical</td><td>$188,006,605</td><td>$220,832,869</td></tr><tr><td>Lifetime educational</td><td>$256,940,831</td><td>$301,942,088</td></tr><tr><td>Lifetime indirect</td><td>$1,127,532,667</td><td>$1,324,181 252</td></tr><tr><td>Total</td><td>$1,900,628,105</td><td>$2,023,206,325</td></tr></table>
• cfDNA screening dominated the integrated test only when evaluated from a societal perspective.
• ICERs from other perspectives: payer, $344,440; health sector, $270,004; governmental, $167,960</td></tr>
</table>

<table>
<tr><th>Citation
Setting
NCT Number</th><th>Design
Test
Comparator(s)</th><th>Population
Analytic Assumptions</th><th>Main Findings</th></tr>
<tr><td></td><td></td><td></td><td>
cfDNA was cost effective when the unit cost of the cfDNA test was below $352 from the government perspective, $256 from the health care perspective, and $216 from the payer perspective.
Impact:
<table>
<tr><th>Outcomes for 1,000,000 Women</th><th>cfDNA</th><th>Integrated</th></tr>
<tr><td>Cases detected</td><td>1,915</td><td>1,474</td></tr>
<tr><td>Cases diagnosed</td><td>1,360</td><td>1,047</td></tr>
<tr><td>Down syndrome live births</td><td>1,039</td><td>1,221</td></tr>
<tr><td>Unnecessary invasive testing</td><td>687</td><td>11,972</td></tr>
<tr><td>Unaffected procedure-related miscarriages</td><td>5</td><td>91</td></tr>
</table>
Cases detected and diagnosed in the first trimester were normalized to account for miscarriage between the first and second trimesters in order to compare screening results.
</td></tr>
<tr><td>Walker et al., 2015[25]

U.S.

Not reported</td><td>Aim:
To determine the optimum MSS risk cutoff for contingent cfDNA screening and compare the cost effectiveness of optimized contingent cfDNA screening to universal cfDNA screening and conventional MSS

Design:
Cost-effectiveness analysis using microsimulation

Test:
cfDNA screening (test not named)</td><td>Population:
Theoretical cohort of 1,000,000 pregnant women, representative of the U.S. general obstetric prenatal screening population

Conditions:
T21, T18, and T13

Analytic assumptions:
• Societal, government, and payer perspectives
• Annual discount rate of 3%
• Second-trimester risk cutoff: T21, 1:270; T18 and T13, 1:100

See Table C18 for other analytic assumptions.</td><td>Cost effectiveness:
See Table C19 for costs and cases detected.

Societal Perspective
• Contingent cfDNA screening detection rate: T21, 93.6%; T18, 92.7%; T13, 77.7%
• Conventional MSS detection rate: T21, 84.4%; T18, 75.8%; T13, 62.8%
• Universal cfDNA screening detection rate: T21, 98.7%; T18, 96.4%; T13, 91.5%
• Contingent cfDNA screening FP rate: 0.09%
• Conventional MSS FP rate: 5.6%
• Universal cfDNA screening FP rate: 0.9%
• Contingent cfDNA screening failure rate: 0.66%
• Universal cfDNA screening FP rate: 2.8%
• Approximately 24% of women were referred for cfDNA screening after primary screening.</td></tr>
</table>

Citation Setting NCT Number	Design Test Comparator(s)	Population Analytic Assumptions	Main Findings
	Comparators: • Contingent cfDNA screening using the combined serum test (PAPP-A, free ß-hCG, and NT) • MSS using the combined serum test • No screening		• Out of 1,000,000 pregnancies, replacing MSS with universal cfDNA screening would result in an increase of 893 detections and a cost savings of approximately $170 million. • Universal cfDNA screening was less costly than conventional MSS if the cost of cfDNA screening was below $619. • In the probabilistic sensitivity analysis, universal cfDNA screening was more effective 100% of the time and less costly 91.1% of the time compared to MSS. Government Perspective • Contingent cfDNA screening detection rate: T21, 87%; T18, 82.1%; T13, 77.7% • Contingent cfDNA screening FP rate: 0.033% • Contingent cfDNA screening failure rate: 0.24% • Approximately 8.7% of women were referred for cfDNA screening after primary screening. • Out of 1,000,000 pregnancies, replacing combined MSS with contingent cfDNA screening would result in an increase of 301 detections and a cost savings of approximately $17.5 million. • Universal cfDNA screening was more effective but also more costly than contingent cfDNA screening. Universal cfDNA screening would increase the number of cases detected by contingent cfDNA screening by 592 and increase costs by $120 million, for an ICER of $203,088 per additional case detected. • Contingent cfDNA screening dominated MSS unless the cost of cfDNA screening exceeded $663. • In the probabilistic sensitivity analysis, contingent screening was more effective 100% of the time and less costly 87% of the time compared to MSS. Payer Perspective • Contingent cfDNA screening detection rate: T21, 85.1%; T18, 75.8%; T13, 63.3% • Contingent cfDNA screening FP rate: 0.026% • Contingent cfDNA screening failure rate: 0.19%

<table>
<tr><th>Citation
Setting
NCT Number</th><th>Design
Test
Comparator(s)</th><th>Population
Analytic Assumptions</th><th>Main Findings</th></tr>
<tr><td></td><td></td><td></td><td>

Approximately 7% of women were referred for cfDNA screening after primary screening.
Compared to no screening, MSS would cost $56,726 per case detected; however, contingent cfDNA screening would cost $54,309 for each detection. Therefore, contingent cfDNA screening dominates MSS by extension.
Compared to contingent cfDNA screening, universal cfDNA screening would increase the number of cases detected by 680 and increase costs by $179 million, for an ICER of $263,922 per additional case detected.
The one-way analysis shows contingent cfDNA screening was less costly than MSS when the cost of cfDNA screening was below $293, when contingent cfDNA screening uptake was below 72%, and when the cost of invasive screening was above $1,235. In the probabilistic sensitivity analysis, contingent screening was more effective 100% of the time and more costly 73.2% of the time compared to MSS.

Impact:

Prevalence at 12 weeks: T21, 1 in 301; T18. 1 in 1,170; T13, 1 in 3,627
Prevalence at birth: T21, 1 in 528; T18. 1 in 4,174; T13, 1 in 7,084

<table>
<tr><th rowspan="2">Perspective</th><th colspan="3">Optimal Risk Cutoff</th><th rowspan="2">cfDNA Screening Referral Rate</th></tr>
<tr><th>T21</th><th>T18</th><th>T13</th></tr>
<tr><td>Societal</td><td>1:1,515</td><td>1:1,905</td><td>1:860</td><td>24.0%</td></tr>
<tr><td>Government</td><td>1:420</td><td>1:145</td><td>1:175</td><td>8.7%</td></tr>
<tr><td>Payer</td><td>1:315</td><td>1:115</td><td>1:175</td><td>7.0%</td></tr>
</table>
See Table C20 for test performance outcomes.
</td></tr>
</table>

Abbreviations. 45,X: Turner syndrome; 47,XXX: Triple X syndrome; 47,XXY: Klinefelter syndrome; 47,XYY: Jacob's syndrome; AFP: alpha-fetoprotein; AMA: advanced maternal age; cfDNA: cell-free DNA; CI: confidence interval; CMA: chromosomal microarray analysis; CMS: Centers for Medicare and Medicaid Services; CVS: chorionic villus sampling; FN: false negative; FP: false positive; FTS: first-trimester screening; hCG: human chorionic gonadotrophin; ICER: incremental cost-effectiveness ratio; MSS: maternal serum screen; NT: nuchal translucency; PAPP-A: pregnancy-associated plasma protein A; QALY: quality-adjusted life year; QUAD: second-trimester quadruple screening; T13: trisomy 13; T18; trisomy 18; T21: trisomy 21.

Table C8. Incidence Rates and Test Performance in the First and Second Trimester

Condition	Outcome	First Trimester	Second Trimester
T21			
Prevalence		1 in 365	1 in 398
Conventional screening	Sensitivity	85.3%	84.1%
	Specificity	95.2%	92.5%
cfDNA screening	Sensitivity	99.3%	99.3%
	Specificity	99.9%	99.9%
T18			
Prevalence		1 in 1,208	1 in 1,487
Conventional screening	Sensitivity	95.0%	73.5%
	Specificity	99.7%	99.8%
cfDNA screening	Sensitivity	96.8%	96.8%
	Specificity	99.9%	99.9%
T13			
Prevalence		1 in 3,745	1 in 4,195
Conventional screening	Sensitivity	94.5%	16.4%
	Specificity	NR	NR
cfDNA screening	Sensitivity	87.2%	87.2%
	Specificity	99.8%	99.8%
45,X			
Prevalence		1 in 1,291	1 in 2,340
Conventional screening	Sensitivity	75.0%	54.1%
	Specificity	NR	NR
cfDNA screening	Sensitivity	89.5%	89.5%
	Specificity	99.8%	99.8%

Abbreviations. 45,X: Turner syndrome; cfDNA: cell-free DNA; NR: not reported; T13: trisomy 13; T18; trisomy 18; T21: trisomy 21. Source. Adapted from Benn et al., 2015.[18]

Table C9. Impact of cfDNA Screening in a Theoretical Cohort of 3,952,841 Live Births

Outcome	Conventional Screening	cfDNA Screening
T21		
Affected pregnancies screened	7,836	7,836
T21 affected with positive result	6,687	7,783
T21 births averted	2,901	4,097
Invasive tests	53,813	9,010
Procedure-related euploid losses	246	13
T18		
Affected pregnancies screened	9,010	9,010
T18 affected with positive result	2,246	2,288
T18 births averted	426	436
Invasive tests	5,604	4,282
Procedure-related euploid losses	18	12
T13		
Affected pregnancies screened	763	763
T13 with positive result	721	665
T13 births averted	293	268
Invasive tests	614	4,624
Procedure-related euploid losses	0	20
45,X		
Affected pregnancies screened	2,214	2,214
45,X affected with positive result	1,660	1,981
45,X births averted	9	41
Invasive tests	1,399	6,680
Procedure-related euploid losses	0	25
All Aneuploidies Combined		
Affected pregnancies screened	13,176	13,176
Affected with positive result	11,314	12,717
Affected births averted	3,629	4,842
Invasive tests	61,430	24,596
Procedure-related euploid losses	264	70

Abbreviations. 45,X: Turner syndrome; cfDNA: cell-free DNA; T13: trisomy 13; T18; trisomy 18; T21: trisomy 21. Source. Adapted from Benn et al., 2015.[18]

Table C10. Costs per Patient Screened in Base Case and Alternative Scenarios

Strategy	Conventional Screening Protocol							
	Cost per Patient ($)				Marginal Cost per Additional Case (million $)			
	Free ß-hCG + PAPP-A + NT	ß-hCG + PAPP-A + NT	Free ß-hCG + PAPP-A + NT + NB	ß-hCG + PAPP-A + NT + NB	Free ß-hCG + PAPP-A + NT	ß-hCG + PAPP-A + NT	Free ß-hCG + PAPP-A + NT + NB	ß-hCG + PAPP-A + NT + NB
Base Case								
Hybrid	474	530	386	406	5.1	3.7	11.6	9.2
Contingent 1/300 cut-off	430	515	300	332	4.1	3.0	9.2	7.2
Contingent 1/1,000 cut-off	409	487	291	320	7.3	5.0	16.0	12.2
Primary cfDNA	1,017	1,017	1,017	1,017	Reference	Reference	Reference	Reference
cfDNA FP Rate = 0.1% and TP Rate = 99.9%								
Hybrid	464	520	375	395	5.1	3.7	11.5	9.2
Contingent 1/300 cut-off	420	505	290	321	4.1	3.0	9.2	7.2
Contingent 1/1,000 cut-off	398	476	280	310	7.2	5.0	15.9	12.1
Primary cfDNA	1,005	1,005	1,005	1,005	Reference	Reference	Reference	Reference
30% Patients Decline Invasive Testing								
Hybrid	988	1,029	923	939	7.3	5.3	16.6	13.2
Contingent 1/300 cut-off	927	989	829	853	5.9	4.3	13.2	10.3
Contingent 1/1,000 cut-off	934	1,001	833	857	10.4	7.2	22.9	17.4
Primary cfDNA	1,573	1,573	1,573	1,573	Reference	Reference	Reference	Reference
30% Detected Cases Not Terminated								
Hybrid	989	1,020	924	940	7.3	5.3	16.6	13.2
Contingent 1/300 cut-off	927	989	830	854	5.9	4.3	13.2	10.3
Contingent 1/1,000 cut-off	935	1,002	833	858	10.4	7.2	22.9	17.4
Primary cfDNA	1,574	1,574	1,574	1,574	Reference	Reference	Reference	Reference
30% Patients Decline cfDNA Testing								
Hybrid	935	975	873	888	4.7	3.4	10.6	8.4

Strategy	Conventional Screening Protocol							
	Cost per Patient ($)				Marginal Cost per Additional Case (million $)			
	Free ß-hCG + PAPP-A + NT	ß-hCG + PAPP-A + NT	Free ß-hCG + PAPP-A + NT + NB	ß-hCG + PAPP-A + NT + NB	Free ß-hCG + PAPP-A + NT	ß-hCG + PAPP-A + NT	Free ß-hCG + PAPP-A + NT + NB	ß-hCG + PAPP-A + NT + NB
Contingent 1/300 cut-off	912	972	821	843	3.8	2.7	8.4	6.6
Contingent 1/1,000 cut-off	899	954	815	836	6.5	4.5	14.6	11.0
Primary cfDNA	1,273	1,273	1,273	1,273	Reference	Reference	Reference	Reference
Cost of NT Included for All Patients								
Hybrid	493	548	404	424	5.9	4.3	13.4	10.6
Contingent 1/300 cut-off	430	515	300	332	4.8	3.5	10.6	8.3
Contingent 1/1,000 cut-off	409	487	291	320	8.5	6.0	18.6	14.1
Primary cfDNA	1,139	1,139	1,139	1,139	Reference	Reference	Reference	Reference
Cost cfDNA = $700								
Hybrid	421	476	336	355	3.3	2.4	7.4	5.9
Contingent 1/300 cut-off	416	498	292	322	2.6	1.9	5.9	4.6
Contingent 1/1,000 cut-off	373	440	274	299	4.6	3.1	10.2	7.7
Primary cfDNA	717	717	717	717	Reference	Reference	Reference	Reference
Additional Cost for Third Biochemical Marker								
Hybrid	492	548	403	424	5.0	3.6	11.3	9.0
Contingent 1/300 cut-off	451	535	321	353	4.0	2.9	9.0	7.0
Contingent 1/1,000 cut-off	429	507	312	341	7.1	4.9	15.6	11.8
Primary cfDNA	1,017	1,017	1,017	1,017	Reference	Reference	Reference	Reference
Clinical Costs Increased by 20%								
Hybrid	496	552	407	428	4.9	3.6	11.3	9.0
Contingent 1/300 cut-off	455	540	326	357	4.0	2.9	9.0	7.0
Contingent 1/1,000 cut-off	436	514	317	346	7.0	4.8	15.5	11.8
Primary cfDNA	1,017	1,017	1,017	1,017	Reference	Reference	Reference	Reference

Abbreviations. cfDNA: cell-free DNA: FP: false positive; hCG: beta human chorionic gonadotrophin; NB: nasal bone; NT: nuchal translucency; PAPP-A: pregnancy-associated plasma protein A; TP: true-positive. Source. Adapted from Evans et al., 2015.[20]

Table C11. Cost per Patient Screened and Marginal Costs Using the Best-Case Scenario for cfDNA Screening

Screening Strategy (Risk Cut-Off)	Conventional Screening Protocol			
	Free ß-hCG + PAPP-A + NT	ß-hCG + PAPP-A + NT	Free ß-hCG + PAPP-A + NT + NB	ß-hCG + PAPP-A + NT + NB
Cost per Patient Screened				
Hybrid (1/300)	$930	$969	$869	$883
Contingent (1/300)	$941	$999	$854	$875
Contingent (1/1,000)	$914	$962	$842	$860
Primary cfDNA	$1,055	$1,055	$1,055	$1,055
Marginal Cost of Primary cfDNA Screening				
Hybrid (1/300)	$2.4 million	$1.7 million	$5.5 million	$4.3 million
Contingent (1/300)	$1.9 million	$1.4 million	$4.3 million	$3.4 million
Contingent (1/1,000)	$3.1 million	$2.1 million	$7.3 million	$5.5 million

Abbreviations. cfDNA: cell-free DNA: hCG: beta human chorionic gonadotrophin; NB: nasal bone; NT: nuchal translucency; PAPP-A: pregnancy-associated plasma protein A. Source. Adapted from Evans et al., 2015.[20]

Table C12. Expected Numbers of Patients Undergoing Testing in a Population of 1 Million

Strategy	Conventional Screening Protocol			
	Free ß-hCG + PAPP-A + NT	ß-hCG + PAPP-A + NT	Free ß-hCG + PAPP-A + NT + NB	ß-hCG + PAPP-A + NT + NB
Primary cfDNA Screening Strategy				
Conventional screens	0	0	0	0
Positive results	0	0	0	0
cfDNA screens	1,000,000	1,000,000	1,000,000	1,000,000
Positive results	4,027	4,027	4,027	4,027
Missed cases of viable down syndrome	12	12	12	12
Hybrid Strategy				
Conventional screens	851,482	851,482	851,482	851,482
Positive results	26,479	30,235	17,123	21,657
cfDNA screens	176,251	179,737	166,624	171,158
Positive results	2,542	2,462	2,612	2,598
Missed cases of viable down syndrome	146	196	72	87
Contingent Strategy with 1/300 Cut-off				

Strategy	Conventional Screening Protocol			
	Free ß-hCG + PAPP-A + NT	ß-hCG + PAPP-A + NT	Free ß-hCG + PAPP-A + NT + NB	ß-hCG + PAPP-A + NT + NB
Conventional screens	1,000,000	1,000,000	1,000,000	1,000,000
Positive results	47,094	57,121	27,229	33,738
cfDNA screens	47,094	57,121	27,229	33,738
Positive results	2,240	2,160	2,350	2,328
Missed cases of viable down syndrome	203	273	100	123
Contingent Strategy with 1/1,000 Cut-off				
Conventional screens	1,000,000	1,000,000	1,000,000	1,000,000
Positive results	118,025	155,893	57,232	70,287
cfDNA screens	118,025	155,893	57,232	70,287
Positive results	2,481	2,493	2,452	2,453
Missed cases of viable down syndrome	110	145	61	75

Abbreviations. cfDNA: cell-free DNA: hCG: beta human chorionic gonadotrophin; NB: nasal bone; NT: nuchal translucency; PAPP-A: pregnancy-associated plasma protein A.
Source. Adapted from Evans et al., 2015.[20]

Table C13. Key Probabilities Used in the Analysis

Outcome	Value
cfDNA Failed Test: No Results Returned	
Probability of failed cfDNA when sex chromosome aneuploidy is present	.07
Probability of failed cfDNA when T13 is present	.15
Probability of failed cfDNA when T18 is present	.11
Probability of failed cfDNA when T21 is present	.04
Probability of failed cfDNA in the absence of aneuploidy	.04
cfDNA Test Characteristics When a Result is Returned	
Sensitivity of cfDNA for T13	.92
Sensitivity of cfDNA for T18	.97
Sensitivity of cfDNA for T21	.99
Sensitivity of cfDNA for sex chromosome aneuploidy	.91
False-positive rate for cfDNA	.007

Outcome	Value
Probability of Additional Testing	
Probability of diagnostic testing with a negative screening test	.004
Probability of diagnostic testing after positive multiple marker screening when cfDNA is available	.39
Probability of cfDNA testing after positive multiple marker screening	.39
Probability of diagnostic testing after positive multiple marker screening when cfDNA is not available	.78
Probability of diagnostic testing after cfDNA positive for trisomy or no result returned	.78
Probability of Termination	
Probability of termination for T13	.65
Probability of termination for T18	.60
Probability of termination for T21	.74
Probability of termination for microarray or rare chromosome abnormality	.74
Probability of termination for variant of uncertain clinical significance	.33
Probability of termination for sex chromosome aneuploidy	.33
Probability of Pregnancy Loss	
Probability of procedure-related loss	.003
Probability of spontaneous loss with T13	.42
Probability of spontaneous loss with T18	.72
Probability of spontaneous loss with T21	.04
Probability of Future Birth After Pregnancy Loss	
Age 30 years and younger	.75
Age 35 years	.66
Age 40 years	.44
Age-Independent Probabilities	
Probability of clinically significant microarray abnormality or rare chromosomal abnormality	.011
Probability of variant of unknown clinical significance	.013

Outcome	Value
Age-Dependent Probabilities	
Age 20	
T13	.0001
T18	.0002
T21	.0008
Sex chromosome aneuploidy (XXX, XXY, XYY, XO)	.003
Age 25	
T13	.0001
T18	.0002
T21	.001
Sex chromosome aneuploidy (XXX, XXY, XYY, XO)	.003
Age 30	
T13	.0002
T18	.0004
T21	.0014
Sex chromosome aneuploidy (XXX, XXY, XYY, XO)	.003
Age 35	
T13	.0004
T18	.0009
T21	.003
Sex chromosome aneuploidy (XXX, XXY, XYY, XO)	.004
Age 40	
T13	.001
T18	.003
T21	.001
Sex chromosome aneuploidy (XXX, XXY, XYY, XO)	.005

Abbreviations. cfDNA: cell-free DNA; T13: trisomy 13; T18: trisomy 18; T21: trisomy 21. Source. Adapted from Kaimal et al, 2015.[22]

Table C14. Quality-Adjusted Life-Years and Costs per 100,000 Women

Testing Strategy	Quality-Adjusted Life-Years	Costs for Testing and Care Until End of the Pregnancy	Incremental Cost-Effectiveness Ratio (Cost/QALY)
Age 20			
MMS,[a] diagnostic testing as follow-up	2,749,610	$912,200,000	Dominant[b]
MMS, diagnostic testing or cfDNA as follow-up	2,749,050	$913,800,000	Dominated[c]
cfDNA, diagnostic testing as follow-up	2,748,560	$1,030,600,000	Dominated
cfDNA and NT, diagnostic testing as follow-up	2,748,310	$1,066,000,000	Dominated
cfDNA and MMS concurrently, diagnostic testing as follow-up	2,748,780	$1,099,600,000	Dominated
Diagnostic testing without prior screening	2,749,280	$1,069,900,000	Dominated
Age 30			
MMS, diagnostic testing as follow-up	2,561,480	$913,800,000	Dominant
MMS, diagnostic testing or cfDNA as follow-up	2,560,950	$915,200,000	Dominated
cfDNA, diagnostic testing as follow-up	2,560,460	$1,030,400,000	Dominated
cfDNA and NT, diagnostic testing as follow-up	2,560,250	$1,065,600,000	Dominated
cfDNA and MMS concurrently, diagnostic testing as follow-up	2,560,690	$1,099,300,000	Dominated
Diagnostic testing without prior screening	2,560,890	$1,069,400,000	Dominated
Age 40			
MMS, diagnostic testing as follow-up	2,322,690	$942,000,000	$1,992, compared with the least expensive strategy
MMS, diagnostic testing or cfDNA as follow-up	2,322,400	$941,500,000	Least expensive strategy
cfDNA, diagnostic testing as follow-up	2,323,840	$1,026,000,000	$73,154
cfDNA and NT, diagnostic testing as follow-up	2,323,170	$1,061,100,000	Dominated
cfDNA and MMS concurrently, diagnostic testing as follow-up	2,323,510	$1,094,800,000	Dominated
Diagnostic testing without prior screening	2,321,610	$1,060,800,000	Dominated

Abbreviations. cfDNA: cell-free DNA; MMS: multiple-marker screening; NT: nuchal translucency; QALY: quality-adjusted life year. Notes. [a] MMS included NT, first-trimester human chorionic gonadotropin and pregnancy-associated placental protein A and second-trimester a-fetoprotein, estriol, human chorionic gonadotropin, and inhibin. [b] A dominant strategy is more effective (results in higher numbers of QALYs) and less costly than the other strategies. A cost-effectiveness ratio was not generated for dominant strategies because they are cost-saving. c A dominated strategy is one that is more costly and less effective than the strategy to which it is being compared. Cost-effectiveness ratios for these strategies were not generated as there is a net decrement—not increment—in effectiveness. Source. Adapted from Kaimal et al, 2015.[22]

Table C15. Pregnancy Outcomes per 1,000 Women

Testing Strategy	True Positive Screening Test		Diagnostic Procedures	Cases of Fetal Abnormality Missed	Procedure-Related Losses	Procedures/Case Diagnosed
	Any Fetal Abnormality	Fetal T21				
Age 20						
MMS, diagnostic testing as follow-up	1,612	65	7,073	1,582	18	5.4
MMS, diagnostic testing or cfDNA as follow-up	1,612	65	5,818	1,863	15	5.7
cfDNA, diagnostic testing as follow-up	363	79	7,509	2,530	18	20.9
cfDNA and NT, diagnostic testing as follow-up	1,388	80	9,498	2,163	24	13.1
cfDNA and MMS concurrently, diagnostic testing as follow-up	1,749	80	9,617	2,044	24	11.4
Diagnostic testing without prior screening	NR	NR	100,000	0	250	34.6
Age 30						
MMS, diagnostic testing as follow-up	1,689	118	7,909	1,612	20	5.8
MMS, diagnostic testing or cfDNA as follow-up	1,689	118	6,286	1,883	16	5.7
cfDNA, diagnostic testing as follow-up	601	139	7,640	2,483	19	15.4
cfDNA and NT, diagnostic testing as follow-up	1,562	139	9,597	2,096	24	11.5
cfDNA and MMS concurrently, diagnostic testing as follow-up	1,895	140	9,715	2,028	24	10.2
Diagnostic testing without prior screening	NR	NR	100,000	0	250	33.6
Age 40						
MMS, diagnostic testing as follow-up	3,138	1,114	22,767	2,054	57	9.1
MMS, diagnostic testing or cfDNA as follow-up	3,138	1,114	14,320	2,456	36	6.8
cfDNA, diagnostic testing as follow-up	2,132	1,149	8,674	2,884	22	5.3
cfDNA and NT, diagnostic testing as follow-up	3,114	1,155	10,599	2,573	26	5.4
cfDNA and MMS concurrently, diagnostic testing as follow-up	3,138	1,160	10,744	2,456	27	5.1

Diagnostic testing without prior screening	NR	NR	100,000	0	250	22.0

Abbreviations. cfDNA: cell-free DNA; MMS: multiple-marker screening; NR: not relevant; NT: nuchal translucency; T21: trisomy 21. Note. MMS included NT, first-trimester human chorionic gonadotropin and pregnancy-associated placental protein A and second-trimester a-fetoprotein, estriol, human chorionic gonadotropin, and inhibin. Source. Adapted from Kaimal et al, 2015.[22]

Table C16. Calculated Costs of Screening for 3,000 Women

Strategy	Cost of Initial Screen (74.5% uptake)	Cost of Diagnostic Test (100% uptake)	Overall Cost of Test	Marginal Additional Cost of Additional Aneuploidy Detected
Down Syndrome				
Universal cfDNA Screening	$1,341,000	$5,064	$1,346,064	$1,101,179
Sequential	$207,855	$37,030	$244,885	Reference
All Aneuploidies Detectable				
Universal cfDNA screening	$1,341,000	$12,660	$1,353,660	Reference
Sequential	$207,855	$31,334	$239,189	Saving of $1,114,471

Abbreviation. cfDNA: cell-free DNA. Source. Adapted from Shiv et al., 2017.[23]

Table C17. Model Probabilities and Costs

Assumption	Baseline Estimate	Range
Model Probabilities		
Uptake of integrated screen	67%	63% to 71%
Uptake of cfDNA screening	81%	69% to 92%
Uptake of invasive/diagnostic testing	71%	67% to 76%
Termination rate for positive diagnosis	75%	60% to 90%
Procedure-related fetal loss	0.75%	0.5% to 1%
Spontaneous fetal loss of down syndrome pregnancies from first trimester to term	43%	Not defined
Spontaneous fetal loss of down syndrome pregnancies from second trimester to term	23%	Not defined
Costs		
Integrated (first-trimester markers)	$145	$73 to $290
Integrated (second-trimester markers)	$98	$49 to $196
cfDNA	$400	$200 to $800
Diagnostic testing	$1,100	$550 to $2,200
Genetic counseling (after positive diagnostic test)	$160	$80 to $320
First-trimester termination	$581	$291 to $1,162

Second-trimester termination	$1,673	$837 to $3,346
Lifetime costs associated with Down syndrome	$1,496,772	$748,386 to $2,993,544

Abbreviation. cfDNA: cell-free DNA. Source. Adapted from Walker et al., 2015.[24]

Table C18. Model Probabilities and Costs

Assumption	Mean	95% CI
Model Probabilities		
MSS uptake	69%	64% to 74%
Increase in contingent cfDNA screening uptake over MSS	8.2%	4.6% to 12.6%
Increase in universal cfDNA screening uptake over MSS	13.5%	7.6% to 20.8%
Diagnostic testing uptake	66%	61% to 71%
Procedure-related fetal loss	0.22%	0% to 1.16%
Termination rate of T21	80%	74% to 86%
Termination rate of T18	80%	73% to 87%
Termination rate of T13	92%	85% to 97%
cfDNA screening detection rate (sensitivity) of T21	99%	98.3% to 99.5%
cfDNA screening detection rate (sensitivity) of T18	96.8%	95% to 98.2%
cfDNA screening detection rate (sensitivity) of T13	92.1%	86.9% to 96.1%
cfDNA screening FP rate	0.41%	0.29% to 0.55%
cfDNA screening failure rate due to low fetal fraction	2.8%	1.2% to 5.1%
Costs		
Combined screen	$166	$95 to $257
cfDNA screening	$400	$229 to $619
CVS	$1,010	$577 to $1,562
Genetic counseling	$160	$91 to $247
Termination of pregnancy	$581	$332 to $898
Direct lifetime T21	$427,577	$244,397 to $661,147
Indirect lifetime T21	$1,069,195	$611,137 to $1,653,257
Direct lifetime T13 and T18	$37,971	$21,704 to $58,713
Indirect lifetime T13 and T18	$1,363,877	$779,574 to $2,108,913

Abbreviations. cfDNA: cell-free DNA; CVS: chorionic villus sampling; FP: false positive; MSS: maternal serum screen; T13: trisomy 13; T18: trisomy 18; T21: trisomy 21. Source. Adapted from Walker et al., 2015.[25]

Table C19. Costs and Cases Detected in 1,000,000 Women

Strategy	Total Cost	Cases Detected	Incremental Costs	Incremental Cases Detected	ICER
Societal Perspective					
No screening	$3,347,297,152	0	NR	NR	Strictly dominated
MSS	$2,475,580,143	2,516	NR	NR	Strictly dominated
Contingent cfDNA screening	$2,315,959,639	3,077	NR	NR	Strictly dominated
Universal cfDNA screening	$2,305,749,493	3,409	NR	NR	Strictly dominated
Government Perspective					
No screening	$822,000,565	0	NR	NR	Strictly dominated
MSS	$711,465,188	2,516	NR	NR	Strictly dominated
Contingent cfDNA screening	$693,996,197	2,817	NR	NR	Strictly dominated
Universal cfDNA screening	$814,224,159	3,409	$120,277,962	592	$203,088
Payer Perspective					
No screening	$0	0	NR	NR	NR
MSS	$142,723,273	2,516	NR	NR	Dominated by extension
Contingent cfDNA screening	$148,208,927	2,729	$148,208,927	213	$25,754
Universal cfDNA screening	$327,675,783	3,409	$179,466,856	608	$263,922

Abbreviations. cfDNA: cell-free DNA; ICER: incremental cost-effectiveness ratio; MSS: maternal serum screen; NR: not relevant. Source. Adapted from Walker et al., 2015.[25]

Table C20. Test Performance Outcomes

Perspective	Detection Rates (Sensitivities)			FP Rate	cfDNA Screening Failure Rate
	T21	T18	T13		
Universal cfDNA screening	99%	96.8%	92.1%	0.4%	2.8%
MSS	84.8%	75.8%	62.8%	5.6%	0%
Contingent cfDNA screening					
Societal perspective	93.6%	92.7%	77.7%	0.09%	0.66%
Government perspective	87%	82.1%	63.3%	0.03%	0.24%
Payer perspective	85.1%	75.6%	63.3%	0.03%	0.19%

Abbreviations. cfDNA: cell-free DNA; FP: false positive; MSS: maternal serum screen; T13: trisomy 13; T18: trisomy 18; T21: trisomy 21. Source. Adapted from Walker et al., 2015.[25]

Appendix D. Risk of Bias Assessments

Table D1. Risk of Bias: Randomized Controlled Trials

Study	Randomization	Allocation Concealment	Intervention	Outcomes	Investigator & Participant Masking	Outcome Assessor Masking	Intention to Treat Analysis	Statistical Analysis	Other Biases	Interest Disclosure	Funding	Overall Risk of Bias Assessment Comments
Kagan et al., 2018[8]	Yes	Unclear	Yes	Yes	Unclear	Unclear	Yes	Yes	None	Yes	No	**Moderate** Some concern about lack of blinding and allocation concealment

Table D2. Risk of Bias: Diagnostic Test Accuracy Studies

Part 1

Study	Patient Representation	Patient Selection	Reference Standard	Test Timing	Verification	Use of Reference Standard	Test Independence
Ashoor et al., 2013[9]	Unclear	No	Yes	NA	Yes	Yes	Unclear
Bianchi et al., 2014[10]	Yes	Unclear	Yes	NA	Yes	Yes	Yes
del Mar Gil et al., 2014[11]	Yes	No	Yes	NA	Yes	Yes	Unclear
Langlois et al., 2017[12]	Yes	Unclear	Yes	NA	Yes	Yes	Yes
Nicolaides et al., 2012[13]	Yes	Unclear	Yes	NA	Yes	Yes	Yes
Norton et al., 2015[14]	Yes	Unclear	Yes	NA	Yes	Yes	Yes
Palomaki et al., 2017[15,92]	Yes	Unclear	Yes	NA	Yes	Yes	Unclear
Pergament et al., 2014[16]	Yes	Unclear	Yes	NA	Yes	Yes	Yes
Quezada et al., 2015[17]	No	Unclear	Yes	NA	Yes	Yes	Unclear

Abbreviation. NA: not applicable.

Part 2

Study	Interpretation of Index Test	Interpretation of Reference Standard	Uninterpretable or Intermediate Test Results	Withdrawals	Interest Disclosure	Funding Source	Overall Risk of Bias Assessment Comments
Ashoor et al., 2013[9]	Unclear	Unclear	Unclear	Unclear	No	Unclear	**High** Some significant concerns about the case-control design and a general lack of reporting
Bianchi et al., 2014[10]	Yes	Yes	Yes	Unclear	No	No	**Moderate** Some concern about patient selection and conflicts of interest
del Mar Gil et al., 2014[11]	Unclear	Unclear	Yes	Unclear	No	Yes	**Moderate** Some concern about patient selection and conflicts of interest
Langlois et al., 2017[12]	Unclear	Unclear	Yes	Yes	Yes	Yes	**Moderate** Some concern about patient selection and test interpretation
Nicolaides et al., 2012[13]	Yes	Yes	Yes	Unclear	Yes	Yes	**Moderate** Some concern about patient selection and withdrawals
Norton et al., 2015[14]	Yes	Yes	Yes	Yes	No	No	**Moderate** Some concern about conflicts of interest
Palomaki et al., 2017[15,92]	Unclear	Unclear	Yes	Yes	Yes	Yes	**Moderate** Some concern about the lack of reporting around blinding and patient selection
Pergament et al., 2014[16]	Yes	Unclear	Yes	Unclear	No	Unclear	**Moderate** Some concern about the overall lack of reporting and conflicts of interest
Quezada et al., 2015[17]	Unclear	Unclear	Unclear	Unclear	No	Yes	**High** Some significant concerns about patient representation, conflicts of interest, and overall lack of reporting

Table D3. Risk of Bias: Economic Studies

Part 1

Citation	Target Population	Perspective	Time Horizon	Discount Rate	Comparators	Modeling	Effectiveness
Benn et al., 2015[18]	Yes	No	Unclear	No	Yes	Unclear	Yes
Crimmins et al., 2017[19]	Yes	No	No	No	Yes	Unclear	Yes
Evans et al., 2015[20]	Unclear	Yes	Unclear	No	Yes	Unclear	Unclear
Fairbrother et al., 2016[21]	Yes	No	Unclear	No	Yes	No	Yes
Kaimal et al., 2015[22]	Yes	No	Unclear	Yes	Yes	Yes	Yes
Shiv et al., 2017[23]	No	No	No	No	Yes	Yes	Yes
Walker et al., 2015[24]	Yes	Yes	Yes	Yes	Yes	Yes	Unclear
Walker et al., 2015[25]	Yes	Yes	Unclear	Yes	Yes	Yes	Yes

Part 2

Citation	Outcomes	Resource Use/Costs	Uncertainty	Results	Interest Disclosure	Funding Source	Overall Risk of Bias Assessment Comments
Benn et al., 2015[18]	Yes	Unclear	Yes	Yes	No	No	**Moderate** Some concern around the costs (costs of tests were low), the time horizon used (included some lifetime costs), and the details of the model (no diagram was provided)
Crimmins et al., 2017[19]	Yes	Yes	Yes	No	Yes	No	**High** Some significant concern around the model used (only selected arms were provided as figures), the low false-positive rate, low costs of procedure-related losses, and only a 1-way sensitivity analysis
Evans et al., 2015[20]	Yes	Yes	Yes	Yes	Unclear	No	**Moderate** Some concern about limited details on the population, the time horizon (some lifetime costs were included), assumptions favored cfDNA (an explicit assumption)

Citation	Outcomes	Resource Use/Costs	Uncertainty	Results	Interest Disclosure	Funding Source	Overall Risk of Bias Assessment Comments
Fairbrother et al., 2016[21]	Yes	Yes	Yes	Yes	No	No	**Moderate** Some concern about limited detail on the population, the time horizon (some lifetime costs were included), the basis for the model was a prior high-risk-based model, the use of 2-way sensitivity analysis, and conflicts of interest
Kaimal et al., 2015[22]	Yes	Yes	Yes	Yes	Unclear	Unclear	**Moderate** Some concern about time horizon (lifetime costs for the women included, but not for the infant), the inclusion of conditions not of interest, and lack of clarity around conflicts and funding
Shiv et al., 2017[23]	Yes	Yes	No	Yes	Yes	No	**High** Some significant concern around the very limited reporting and lack of sensitivity analysis; also limited to a very specific population
Walker et al., 2015[24]	Yes	Yes	Yes	Yes	Unclear	Yes	**Low** Some concern around the time horizon (some lifetime costs included)
Walker et al., 2015[25]	Yes	Yes	Yes	Yes	Unclear	Yes	**Low** Some concern around the time horizon (some lifetime costs included)

Table D4. Methodological Quality: Guidelines

Guideline Developer Year	Rigor of Development: Evidence	Rigor of Development: Recommendations	Editorial Independence	Scope & Purpose	Stakeholder Involvement	Clarity & Presentation	Applicability	Overall Assessment
American College of Medical Genetics and Genomics[29] 2016	Poor	Poor	Poor	Good	Poor	Good	Poor	**Poor**
American College of Obstetricians and Gynecologists, Society for Maternal–Fetal Medicine[1] 2016	Poor	Poor	Poor	Good	Poor	Good	Poor	**Poor**
Austrian Society of Obstetrics and Gynecology, Austrian Society of Ultrasound in Medicine, Austrian Society of Pre- and Perinatal Medicine, Austrian Society of Human Genetics, German Society of Ultrasound in Medicine, Fetal Medicine Foundation Germany, Swiss Society of Ultrasound in Medicine[35] 2015	Poor	Poor	Poor	Good	Fair	Good	Fair	**Poor**
Chromosome Abnormality Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis [27] 2015	Poor	Poor	Poor	Good	Fair	Good	Good	**Poor**
European Society of Human Genetics, American Society of Human Genetics[28] 2015	Poor	Poor	Fair	Fair	Fair	Good	Fair	**Poor**
Human Genetics Society of Australia, Royal Australian and New Zealand College of Obstetricians and Gynaecologists[30] 2018	Good	Fair	Fair	Good	Fair	Good	Fair	**Good**
International Society of Ultrasound in Obstetrics and Gynecology[34]	Poor	Poor	Poor	Good	Fair	Good	Fair	**Poor**

Guideline Developer Year	Rigor of Development: Evidence	Rigor of Development: Recommendations	Editorial Independence	Scope & Purpose	Stakeholder Involvement	Clarity & Presentation	Applicability	Overall Assessment
2017								
Israeli Society of Medical Genetics NIPT Committee[31] 2014	Poor	Poor	Poor	Fair	Poor	Fair	Fair	**Poor**
National Society of Genetic Counselors[32] 2016	Poor	Poor	Poor	Poor	Poor	Fair	Fair	**Poor**
Polish Gynecological Society, Polish Human Genetics Society[36] 2017	Poor	Poor	Poor	Good	Fair	Good	Poor	**Poor**
Public Health England and the U.K. National Screening Programme[33] 2015	Good	Good	Good	Good	Good	Fair	Fair	**Good**
Society for Maternal-Fetal Medicine, 2017[37]	Poor	Poor	Fair	Good	Poor	Good	Poor	**Poor**
Society of Obstetricians and Gynaecologists of Canada (SOGC) Genetics Committee, Canadian College of Medical Geneticists (CCMG) Clinical Practice Committee[26] 2017	Fair	Poor	Poor	Good	Poor	Good	Good	**Fair**

Appendix E. GRADE Quality of Evidence

Effectiveness and Harms

Table E1. GRADE Profile: Effectiveness and Harms

Number of Participants and Studies	Risk of Bias	Inconsistency	Indirectness	Imprecision	Publication Bias	Comments	Effect	Overall Quality of Evidence Rating
Outcome: FP Rate for T21								
N = 1,400 1 RCT[8]	Serious (-1) See Risk of Bias assessment	Not serious Not assessable as only 1 study	Not serious	Serious (-1) Wide CIs	Not assessed	Downgraded 1 level each for risk of bias and imprecision (i.e., wide CIs)	cfDNA testing has a significantly lower FP screening rate than has conventional FTS (0% vs. 2.5%; *P* value not reported)	⨁⨁◯◯ LOW
Outcome: Test Failures								
N = 30,238 1 RCT, 8 cohort studies, and 1 case-controlled/cohort study)[8-17]	Serious (-1) See Risk of Bias assessment	Serious (-1) Range, 0.9% to 8.5%	Not serious	Serious (-1) Not assessable	Not assessed	Downgraded 1 level each for risk of bias, inconsistency, and imprecision (i.e., not assessable)	Median 2.8% (range, 0.9% to 8.5%)	⨁◯◯◯ VERY LOW
Outcome: Invasive Testing								
N = 1,400 1 RCT[8]	Serious (-1) See Risk of Bias assessment	Not serious (not assessable as only 1 study)	Not serious	Serious (-1) Not assessable	Not assessed	Downgraded 1 level each for risk of bias and imprecision (i.e., not assessable)	Overall, 1.7% (12/688) of women in the FTS group and 0.3% (2/688) of women in the cfDNA plus ultrasound	⨁⨁◯◯ LOW

Number of Participants and Studies	Risk of Bias	Inconsistency	Indirectness	Imprecision	Publication Bias	Comments	Effect	Overall Quality of Evidence Rating
							group opted for invasive testing	
N = 3,117 2 cohort studies[10,12]	Serious (-1) See Risk of Bias assessment	Not serious	Serious (-1) Based on author estimates, not observed effects	Serious (-1) Not assessable	Not assessed	Downgraded 1 level each for risk of bias, indirectness (author estimates, not observed effects), and imprecision (i.e., not assessable)	cfDNA screening is associated with lower rates of invasive testing	⨁◯◯◯ VERY LOW

Abbreviations. cfDNA: cell-free DNA; CI: confidence interval; FP: false positive; FTS: first-trimester screening; RCT: randomized controlled trial; T21: trisomy 21.

Test Performance

All Common Trisomies (T21, T18, and T13) (excluding studies with twins only)

Table E2. GRADE Profile: Test Accuracy of cfDNA Tests for All Common Trisomies (T21, T18, and T13)

Sensitivity	0.91 to 1.00
Specificity	0.99 to 1.00

Outcome	No. of Studies and Participants	Study Design	Factors That May Decrease Certainty of Evidence					Effect per 1,000 Tested (Range)			Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test Prob-ability of 0.41%	Pre-test Prob-ability of 0.52%	Pre-test Prob-ability of 1.69%	
True Positives (participants with	6 studies[9,10,12,13,15-]	Cross-Sectional	Serious (-1)	Not serious	Not serious	Not serious	Not assessed	4 to 4	5 to 6	15 to 17	

Outcome	No. of Studies and Participants	Study Design	Factors That May Decrease Certainty of Evidence					Effect per 1,000 Tested (Range)			Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test Prob-ability of 0.41%	Pre-test Prob-ability of 0.52%	Pre-test Prob-ability of 1.69%	
common aneuploidies (T21, T18, T13))	[17] 10,856 participants	(cohort type accuracy study) and case-controlled study	See Risk of Bias assessment								⊕⊕⊕◯ MODERATE
False Negatives (participants incorrectly classified as not having common aneuploidies (T21, T18, T13))								0 to 0	0 to 1	0 to 2	
True Negatives (participants without common aneuploidies (T21, T18, T13))	6 studies[9,10,12,13,15-17] 10,856 participants	Cross-Sectional (cohort type accuracy study) and case-controlled study	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	990 to 996	988 to 994	977 to 983	⊕⊕⊕◯ MODERATE
False Positives (participants incorrectly classified as having common aneuploidies (T21, T18, T13))								0 to 6	0 to 6	0 to 6	

Abbreviations. CoE: certainty of evidence; T13: trisomy 13; T18: trisomy 18; T21: trisomy 21.

Table E3. GRADE Profile: Test Performance (PPV and NPV) of cfDNA Tests for All Common Trisomies (T21, T18, and T13)

Outcome	No. of Studies and Participants	Study Design	Factors That May Decrease Certainty of Evidence					Median (Range)	Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias		
PPV	6 studies[9,10,12,13,15-17] 10,856 participants	Cross-sectional (cohort type accuracy study) and case-controlled study	Serious (-1) See Risk of Bias assessment	Not serious	Serious (-1) Range, 40.00% to 100%	Serious (-1) See Table 8	Not assessed	79.7% (40.00% to 100%)	⨁◯◯◯ VERY LOW
NPV	6 studies[9,10,12,13,15-17] 10,856 participants	Cross-sectional (cohort type accuracy study) and case-controlled study	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	100% (99.8% to 100%)	⨁⨁⨁◯ MODERATE

Abbreviations. CoE: certainty of evidence; NPV: negative predictive value; PPV: positive predictive value.

Table E4. GRADE Profile: Test Accuracy of Conventional Screening for All Common Trisomies (T21, T18, and T13)

Sensitivity	1.00 (95% CI, 0.93 to 1.00)
Specificity	0.96 (95% CI, 0.95 to 0.96)

Outcome	No. of Studies and Participants	Study Design	Factors That May Decrease Certainty of Evidence					Effect per 1,000 Tested		Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test Probability of 1.73% (95% CI)	Pre-test Probability of 0.52 (95% CI)	
True Positives (participants with common aneuploidies (T21, T18, T13))	1 study[17] 2,836 participants	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	17 (16 to 17)	5 (5 to 5)	⨁⨁⨁◯ MODERATE
False Negatives (participants incorrectly classified as not having								0 (0 to 1)	0 (0 to 0)	

Outcome	No. of Studies and Participants	Study Design	Factors That May Decrease Certainty of Evidence					Effect per 1,000 Tested		Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test Probability of 1.73% (95% CI)	Pre-test Probability of 0.52 (95% CI)	
common aneuploidies (T21, T18, T13))										
True Negatives (participants without common aneuploidies (T21, T18, T13))	1 study[17] 2,836 participants	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	939 (931 to 946)	939 (931 to 946)	⨁⨁⨁◯ MODERATE
False Positives (participants incorrectly classified as having common aneuploidies (T21, T18, T13))								44 (37 to 52)	44 (37 to 52)	

Abbreviations. CI: confidence interval; CoE: certainty of evidence; T13: trisomy 13; T18: trisomy 18; T21: trisomy 21.

Table E5. GRADE Profile: Test Performance (PPV and NPV) of Conventional Screening for All Common Trisomies (T21, T18, and T13)

Outcome	No. of Studies and Participants	Study Design	Factors That May Decrease Certainty of Evidence					Effect (95% CI)	Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias		
PPV	1 study[17] 2,836 participants	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious Not assessable as only 1 study	Not serious	Not assessed	29.32% (24.96% to 31.94%)	⨁⨁⨁◯ MODERATE
NPV	1 study[17] 2,836 participants	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious (Not assessable as only 1 study)	Not serious	Not assessed	100% (NA)	⨁⨁⨁◯ MODERATE

Abbreviations. CI: confidence interval; CoE: certainty of evidence; NA: not applicable; NPV: negative predictive value; PPV: positive predictive value; T13: trisomy 13; T18: trisomy 18; T21: trisomy 21.

T21 (excluding studies with twins only)

Table E6. GRADE Profile: Test Accuracy of cfDNA Tests for T21

Sensitivity	1.00 to 1.00
Specificity	1.00 to 1.00

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Effect per 1,000 Tested (Range)			Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test Probability of 0.21%	Pre-test Probability of 0.28%	Pre-test Probability of 1.15%	
True Positives (participants with T21)	26,697 7 studies[10,12-17]	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	2 to 2	3 to 3	11 to 12	⨁⨁⨁◯ MODERATE
False Negatives (participants incorrectly classified as not having T21)								0 to 0	0 to 0	-1 to 1	
True Negatives (participants without T21)	26,697 7 studies[10,12-17]	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	995 to 998	994 to 997	985 to 989	⨁⨁⨁◯ MODERATE
False Positives (participants incorrectly classified as having T21)								0 to 3	0 to 3	-1 to 4	

Abbreviations. CoE: certainty of evidence; T21: trisomy 21.

Table E7. GRADE Profile: Test Performance (PPV and NPV) of cfDNA Tests for T21

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Median (Range)	Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias		
PPV	26,697 7 studies[10,12-17]	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Serious (-1) Range, 45.45% to 100%	Serious (-1) See Table 6	Not assessed	96.97% (45.45% to 100%)	⊕◯◯◯ VERY LOW
NPV	26,697 7 studies[10,12-17]	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	100% (all 100%)	⊕⊕⊕◯ MODERATE

Abbreviations. CoE: certainty of evidence; NPV: negative predictive value; PPV: positive predictive value.

Table E8. GRADE Profile: Test Accuracy of Conventional Screening for T21

Sensitivity	0.79 to 1.00
Specificity	0.95 to 0.96

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Effect per 1,000 Tested (Range)				Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test Prob-ability of 0.16%	Pre-test Prob-ability of 0.24%	Pre-test Prob-ability of 0.52%	Pre-test Prob-ability of 0.28%	
True Positives (participants with T21)	18,918[10,12,14] 3 studies	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Serious (-1) Range, 78.95% to 100%	Serious (-1) See Table 9	Not assessed	1 to 2	2 to 2	4 to 5	2 to 3	⊕◯◯◯ VERY LOW
False Negatives (participants incorrectly								0 to 1	0 to 0	0 to 1	0 to 1	

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Effect per 1,000 Tested (Range)				Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test Prob-ability of 0.16%	Pre-test Prob-ability of 0.24%	Pre-test Prob-ability of 0.52%	Pre-test Prob-ability of 0.28%	
classified as not having T21)												
True Negatives (participants without T21)	18,918[10,12,14] 3 studies	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	944 to 962	943 to 962	941 to 959	943 to 961	⨁⨁⨁◯ MODERATE
False Positives (participants incorrectly classified as having T21)								36 to 54	36 to 55	36 to 54	36 to 54	

Abbreviations. CoE: certainty of evidence; T21: trisomy 21.

Table E9. GRADE Profile: Test Performance (PPV and NPV) of Conventional Screening for T21

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Median (Range)	Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias		
PPV	18,918[10,12,14] 3 studies	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	4.17% (3.39% to 7.35%)	⨁⨁⨁◯ MODERATE
NPV	18,918[10,12,14] 3 studies	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	99.95% (99.91% to 100%)	⨁⨁⨁◯ MODERATE

Abbreviations. CoE: certainty of evidence; NPV: negative predictive value; PPV: positive predictive value.

T18 (excluding studies with twins only)

Table E10. GRADE Profile: Test Accuracy of cfDNA Tests for T18

Sensitivity	0.90 to 1.00
Specificity	1.00 to 1.00

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Effect per 1,000 Tested (Range)				Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication bias	Pre-test Prob-ability of 0%	Pre-test Prob-ability of 0.1%	Pre-test Prob-ability of 0.42%	Pre-test Prob-ability of 0.1%	
True Positives (participants with T18)	26,697 participants 7 studies[10,12-17]	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	0 to 0	1 to 1	4 to 4	1 to 1	⨁⨁⨁◯ MODERATE
False Negatives (participants incorrectly classified as not having T18)								0 to 0	0 to 0	0 to 0	0 to 0	
True Negatives (participants without T18)	26,697 participants 7 studies[10,12-17]	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	998 to 1000	997 to 999	994 to 996	993 to 996	⨁⨁⨁◯ MODERATE
False Positives (participants incorrectly classified as having T18)								0 to 2	0 to 2	0 to 2	3 to 6	

Abbreviations. CoE: certainty of evidence; T18: trisomy 18.

Table E11. GRADE Profile: Test Performance (PPV and NPV) of cfDNA Tests for T18

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Median (Range)	Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias		
PPV	26,697 participants 7 studies[10,12-17]	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Serious (-1) Range, 40% to 100%	Serious (-1) See Table 6	Not assessed	77.14% (40.00% to 100%)	⨁◯◯◯ VERY LOW
NPV	26,697 participants 7 studies[10,12-17]	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	100% (99.96% to 100%)	⨁⨁⨁◯ MODERATE

Abbreviations. CoE: certainty of evidence; NPV: negative predictive value; PPV: positive predictive value.

Table E12. GRADE Profile: Test Accuracy of Conventional Screening for T18

Sensitivity	0.80 to 1.00
Specificity	0.99 to 1.00

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Effect per 1,000 Tested (Range)			Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test Probability of 0%	Pre-test Probability of 0.05%	Pre-test Probability of 0.06%	
True Positives (participants with T18)	18,912[10,12,14] 3 studies	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Serious (-1) Range, 80.00% to 100%	Serious (-1) See Table 6	Not assessed	0 to 0	0 to 1	0 to 1	⨁◯◯◯ VERY LOW
False Negatives (participants incorrectly classified as not having T18)								0 to 0	-1 to 1	0 to 1	

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Effect per 1,000 Tested (Range)			Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test Probability of 0%	Pre-test Probability of 0.05%	Pre-test Probability of 0.06%	
True Negatives (participants without T18)	18,912[10,12,14] 3 studies	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	944 to 997	994 to 996	994 to 996	⨁⨁⨁◯ MODERATE
False Positives (participants incorrectly classified as having T18)								3 to 6	4 to 6	3 to 5	

Abbreviations. CoE: certainty of evidence; T18: trisomy 18.

Table E13. GRADE Profile: Test Performance (PPV and NPV) of Conventional Screening for T18

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Median (Range)	Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias		
PPV	18,912[10,12,14] 3 studies	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Serious See Table 9	Not assessed	8.33% (0% to 14.04%)	⨁⨁◯◯ LOW
NPV	18,912[10,12,14] 3 studies	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	100% (99.99% to 100%)	⨁⨁⨁◯ MODERATE

Abbreviations. CoE: certainty of evidence; NPV: negative predictive value; PPV: positive predictive value.

T13 (excluding studies with twins only)

Table E14. GRADE Profile: Test Accuracy of cfDNA Tests for T13

Sensitivity	0.40 to 1.00
Specificity	1.00 to 1.00

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Effect per 1,000 Tested (Range)			Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test Probability of 0%	Pre-test Probability of 0.05%	Pre-test Probability of 0.51%	
True Positives (participants with T13)	22,003 participants 7 studies[9,10,12,14-17]	Cohort & case-controlled type studies	Serious (-1) See Risk of Bias assessment	Not serious	Serious (-1) Range, 40% to 100%	Not serious	Not assessed	0 to 0	0 to 1	2 to 5	⨁⨁◯◯ LOW
False Negatives (participants incorrectly classified as not having T13)								0 to 0	-1 to 1	0 to 3	
True Negatives (participants without T13)	22,003 participants 7 studies[9,10,12,14-17]	Cohort & case-controlled type studies	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	998 to 1000	998 to 1000	993 to 995	⨁⨁⨁◯ MODERATE
False Positives (participants incorrectly classified as having T13)								0 to 2	-1 to 2	0 to 2	

Abbreviations. CoE: certainty of evidence; T13: trisomy 13.

Table E15. GRADE Profile: Test Performance (PPV and NPV) of cfDNA Tests for T13

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Median (Range)	Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias		
PPV	22,003 participants 7 studies[9,10,12,14-17]	Cohort & case-controlled type studies	Serious (-1) See Risk of Bias assessment	Not serious	Serious (-1) Range, 25.00% to 88.89%	Serious (-1) See Table 7	Not assessed	50.0% (25.0% to 88.89%)	⨁◯◯◯ VERY LOW
NPV	22,003 participants 7 studies[9,10,12,14-17]	Cohort & case-controlled type studies	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	100% (99.9% to 100%)	⨁⨁⨁◯ MODERATE

Abbreviations. CoE: certainty of evidence; NPV: negative predictive value; PPV: positive predictive value.

Table E16. GRADE Profile: Test Accuracy of Conventional Screening for T13

Sensitivity	0.50 to 1.00
Specificity	0.99 to 1.00

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Effect per 1,000 Tested (Range)			Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test Probability of 0.02%	Pre-test Probability of 0.11%	Pre-test Probability of 0.05%	
True Positives (participants with T13)	12,084[10,14] 2 studies	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Serious (-1) Range, 50.0% to 100%	Serious (-1) See Table 9	Not assessed	0 to 0	1 to 1	0 to 1	⨁◯◯◯ VERY LOW
False Negatives (participants incorrectly								0 to 0	0 to 0	-1 to 1	

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Effect per 1,000 Tested (Range)			Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test Probability of 0.02%	Pre-test Probability of 0.11%	Pre-test Probability of 0.05%	
classified as not having T13)											
True Negatives (participants without T13)	12,084[10,14] 2 studies	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	993 to 997	992 to 996	993 to 997	⨁⨁⨁◯ MODERATE
False Positives (participants incorrectly classified as having T13)								3 to 7	3 to 7	3 to 7	

Abbreviations. CoE: certainty of evidence; T13: trisomy 13.

Table E17. GRADE Profile: Test Performance (PPV and NPV) of Conventional Screening for T13

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Effect	Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias		
PPV	18,912[10,12,14] 3 studies	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Serious See Table 9	Not assessed	3.45% and 14.29%	⨁⨁◯◯ LOW
NPV	18,912[10,12,14] 3 studies	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious	Not serious	Not assessed	99.99% and 100%	⨁⨁⨁◯ MODERATE

Abbreviations. CoE: certainty of evidence; NPV: negative predictive value; PPV: positive predictive value.

All Common Trisomies (T21, T18, and T13) (studies with twins only)

Table E18. GRADE Profile: Test Accuracy of cfDNA Tests for All Common Trisomies (T21, T18, and T13)

Sensitivity	0.91 (95% CI, 0.59 to 1.00)
Specificity	1.00 (95% CI, 0.98 to 1.00)

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Effect per 1,000 Tested	Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test Probability of 5.73% (95% CI)	
True Positives (participants with common aneuploidies (T21, T18, T13))	192 participants 1 study[11]	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious (not assessable as only 1 study)	Serious (-1) See CIs above	Not assessed	52 (34 to 57)	⨁⨁◯◯ LOW
False Negatives (participants incorrectly classified as not having common aneuploidies (T21, T18, T13))								5 (0 to 23)	
True Negatives (participants without common aneuploidies (T21, T18, T13))	192 participants 1 study[11]	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious (not assessable as only 1 study)	Not serious	Not assessed	943 (924 to 943)	⨁⨁⨁◯ MODERATE
False Positives (participants incorrectly classified as having common aneuploidies (T21, T18, T13))								0 (0 to 19)	

Abbreviations. CI: confidence interval; CoE: certainty of evidence; T13: trisomy 13, T18: trisomy 18; T21: trisomy 21.

Table E19. GRADE Profile: Test Performance (PPV and NPV) of cfDNA Tests for All Common Trisomies (T21, T18, and T13)

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Effect (95% CI)	Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias		
PPV	192 participants 1 study[11]	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious (not assessable as only 1 study)	Not serious	Not assessed	100% (NA)	⨁⨁⨁◯ MODERATE
NPV	192 participants 1 study[11]	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Not serious	Not serious (not assessable as only 1 study)	Not serious	Not assessed	99.45% (96.54% to 99.91%)	⨁⨁⨁◯ MODERATE

Abbreviations. CI: confidence interval; CoE: certainty of evidence; NA: not applicable; NPV: negative predictive value; PPV: positive predictive value.

Sex Chromosome Aneuploidies

Table E20. GRADE Profile: Test Accuracy of cfDNA Tests for Sex Chromosome Aneuploidies

Sensitivity	1.00 (95% CI, 0.16 to 1.00)
Specificity	1.00 (95% CI, 0.99 to 1.00)

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Effect per 1,000 Participants Tested	Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test Probability of 0.42% (95% CI)	
True Positives (participants with SCAs)	474 participants 1 study[16]	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Serious (-1) 45,X only	Not serious Not assessable as only 1 study	Serious (-1) See CIs above	Not assessed	4 (1 to 4)	⨁◯◯◯ VERY LOW
False Negatives (participants								0 (0 to 3)	

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Effect per 1,000 Participants Tested	Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test Probability of 0.42% (95% CI)	
incorrectly classified as not having SCAs)									
True Negatives (participants without SCAs)	474 participants 1 study[16]	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Serious (-1) 45,X only	Not serious Not assessable as only 1 study	Serious (-1) See CIs above	Not assessed	996 (988 to 996)	⨁◯◯◯ VERY LOW
False Positives (participants incorrectly classified as having SCAs)								0 (0 to 8)	

Abbreviations. 45,X: Turner syndrome; CI: confidence interval; CoE: certainty of evidence; SCA: sex chromosome abnormality.

Table E21. GRADE Profile: Test Performance (PPV and NPV) of cfDNA Tests for Sex Chromosome Aneuploidies

Outcome	No. of Participants and Studies	Study Design	Factors That May Decrease Certainty of Evidence					Effect (95% CI)	Test Accuracy CoE
			Risk of Bias	Indirectness	Inconsistency	Imprecision	Publication Bias		
PPV	474 participants 1 study[16]	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Serious (-1) 45,X only	Not serious Not assessable as only 1 study	Not serious	Not assessed	100% (NA)	⨁⨁◯◯ LOW
NPV	474 participants 1 study[16]	Cross-sectional (cohort type accuracy study)	Serious (-1) See Risk of Bias assessment	Serious (-1) 45,X only	Not serious Not assessable as only 1 study	Not serious	Not assessed	100% (NA)	⨁⨁◯◯ LOW

Abbreviations. 45,X: Turner syndrome; CI: confidence interval; CoE: certainty of evidence; NPV: negative predictive value; PPV: positive predictive value.

Cost-Effectiveness

Table E22. GRADE Profile: Cost-Effectiveness

Number of Participants and Studies	Risk of Bias	Inconsistency	Indirectness	Imprecision	Publication Bias	Comments	Effect	Overall Quality of Evidence Rating
Outcome: Cost-Effectiveness								
N > 10,000,000 (women in theoretical cohorts) k = 8 economic studies[18-25]	Serious (-1) See Risk of Bias assessment	Not serious, although there are differences in the findings; these are likely due to model assumptions and comparators	Not serious	Serious (-1) Not assessable	Not assessed		cfDNA is more effective than conventional screening but may be more costly	⨁⨁◯◯ LOW

Abbreviation. cfDNA: cell-free DNA.

Appendix F. Studies Registered at ClinicalTrials.gov

Table F1. Ongoing Studies

Registered Clinical Trial Number (Location)	Title of Study	Study Completion Date[a]	Status of Publications and Whether Study Eligible for Possible Inclusion in Systematic Review
NCT03831256	PErsonalized Genomics for Prenatal Abnormalities Screening USing Maternal Blood (PEGASUS-2): towards first tier screening and beyond	December 2021 (estimated)	No published study; per the RCT protocol, the study would be eligible for this review

Abbreviation. RCT: randomized controlled trial. Note: [a]Study completion date was abstracted from ClinicalTrials.gov.

Appendix G. Measures of Test Performance

True positive (TP): a fetus or infant identified as being at high risk for aneuploidy on screening, confirmed by diagnostic testing and/or birth outcome

False positive (FP): a fetus or infant identified as being at high risk for aneuploidy on screening that is not confirmed by diagnostic testing and/or birth outcome

True negative (TN): a fetus or infant identified as being at low risk for aneuploidy on screening, with euploid status confirmed by diagnostic testing and/or birth outcome

False negative (FN): a fetus or infant identified as being at low risk for aneuploidy on screening, but aneuploidy is confirmed by diagnostic testing and/or birth outcome

Sensitivity (true positive rate): probability that the test will be positive, given the fetus has anueploidy. This is sometimes call the detection rate.

Specificity (true negative rate): probability that the test will be negative, given the fetus does not have aneuploidy.

False-positive rate: the percentage of tests that are incorrectly positive within a population of screened pregnant women

False-negative rate: the percentage of tests that are incorrectly negative within a population of screened pregnant women

$$\textbf{Sensitivity} = \frac{(TP)}{(TP+FN)} \; ; \; \textbf{Specificity} = \frac{(TN)}{(TN+FP)}$$

Screening Test Result	Condition Status, Confirmed by Diagnostic Testing and/or Birth Outcome		Total
	Positive	Negative	
Positive	TP	FP	TP + FP
Negative	FN	TN	FN + TN
Total	TP + FN	FP + TN	TP + FP + FN + TN

Appendix H. See Attachment for Excluded Studies