Clinical Scenario: 28 y/o pregnant female, G1P0 at 10 weeks gestation, presents for her first prenatal visit. She is healthy and has no known risk factors. She asks about available options for genetic screening and wants to understand which tests are most accurate for detecting chromosomal abnormalities in the fetus.

Research Question: In pregnant women in the first trimester, does cell-free DNA testing (noninvasive prenatal testing, NIPT) compared to first-trimester combined screening (serum markers + nuchal translucency) improve detection rates of fetal chromosomal abnormalities such as trisomy 21 while reducing false-positive rates?

PICO Search Terms

P	I	C	O
Pregnancy	Cell-Free Nucleic Acids	First Trimester screening	Chromosomal Abnormalities
First trimester	cell free DNA	Serum screening	aneuploidy
	cfDNA	Nuchal translucency	trisomy 21
	Prenatal Diagnosis	Combined screening	Down syndrome
	NIPT		Sensitivity and Specificity
	noninvasive prenatal testing		false positive rate
			detection rate

Search Strategy:

PubMed:

Search code: ("Pregnancy"[Mesh] OR "First Trimester"[Mesh]) AND ("Cell-Free Nucleic Acids"[Mesh] OR cfDNA[tiab] OR NIPT[tiab] OR "noninvasive prenatal testing"[tiab]) AND ("Nuchal Translucency Measurement"[Mesh] OR "first trimester screening"[tiab] OR "combined screening"[tiab] OR "prenatal screening"[tiab]) AND ("Down Syndrome"[Mesh] OR trisomy 21[tiab] OR aneuploidy[tiab] OR "chromosomal abnormality"[tiab]) AND ("Sensitivity and Specificity"[Mesh] OR "false positive rate"[tiab] OR "detection rate"[tiab] OR “screening”)

Results: 134
Results with filters: 123 (12 years)

Cochrane Library:

Search Terms: ("cell-free DNA" OR cfDNA OR NIPT OR "noninvasive prenatal testing") AND ("first trimester" OR "prenatal screening" OR "nuchal translucency" OR "combined screening") AND ("trisomy 21" OR "Down syndrome" OR aneuploidy OR "chromosomal abnormalities")

Results: 25
Results with filters: 24 (10 years)

Google Scholar:
Search terms: "cell-free DNA" OR NIPT "first trimester screening" OR "nuchal translucency" trisomy 21 OR Down syndrome OR sensitivity OR specificity OR "false positive"

Results: 4,370
Results with filters: 3,410 (10 years)

TRIP Database
Search Terms: ("cell-free DNA" OR NIPT) AND ("first trimester screening" OR "combined screening") AND ("Down syndrome" OR trisomy 21) AND (sensitivity OR specificity OR "false positive")

Results: 99
Results with filters: 85 (10 years)

For my article selection, I aimed to include high-quality evidence that directly compared cell-free DNA (cfDNA/NIPT) screening with first-trimester combined screening (nuchal translucency and serum markers) for detecting fetal aneuploidy, particularly trisomy 21. I prioritized recent studies from the past 10 years and focused on diagnostic accuracy outcomes such as sensitivity, specificity, and false-positive rates.

I found that many studies evaluated either cfDNA or first-trimester screening separately, so I selected only articles that either directly compared both modalities or provided strong real-world or guideline-level evidence relevant to this comparison. All four selected studies were PubMed-indexed and included large multicenter cohorts or systematic evidence reviews.

Two studies (Dar et al., 2022; Norton et al., 2015) provided primary comparative diagnostic performance data, while the other two (Rose et al., 2022; Guy et al., 2019) contributed guideline-level synthesis and U.S.-based real-world cfDNA performance data. I prioritized studies involving U.S. populations or widely applicable clinical data.

Although I searched PubMed, Cochrane, TRIP, and Google Scholar, I ultimately selected all four articles from PubMed because they provided the most relevant and high-quality comparative evidence. Google Scholar was useful for identifying additional papers, but all final studies were verified as PubMed-indexed.

Articles Chosen:

1. Dar, P., Jacobsson, B., MacPherson, C., Egbert, M., Malone, F., Wapner, R. J., Roman, A. S., Khalil, A., Faro, R., Madankumar, R., Edwards, L., Haeri, S., Silver, R., Vohra, N., Hyett, J., Clunie, G., Demko, Z., Martin, K., Rabinowitz, M., Flood, K., … Norton, M. E. (2022). Cell-free DNA screening for trisomies 21, 18, and 13 in pregnancies at low and high risk for aneuploidy with genetic confirmation. *American journal of obstetrics and gynecology*, *227*(2), 259.e1–259.e14. https://doi.org/10.1016/j.ajog.2022.01.019 https://pubmed.ncbi.nlm.nih.gov/35085538/

   Abstract:

Background: Cell-free DNA noninvasive prenatal screening for trisomies 21, 18, and 13 has been rapidly adopted into clinical practice. However, previous studies are limited by a lack of follow-up genetic testing to confirm the outcomes and accurately assess test performance, particularly in women at a low risk for aneuploidy.

Objective: To measure and compare the performance of cell-free DNA screening for trisomies 21, 18, and 13 between women at a low and high risk for aneuploidy in a large, prospective cohort with genetic confirmation of results STUDY DESIGN: This was a multicenter prospective observational study at 21 centers in 6 countries. Women who had single-nucleotide-polymorphism-based cell-free DNA screening for trisomies 21, 18, and 13 were enrolled. Genetic confirmation was obtained from prenatal or newborn DNA samples. The test performance and test failure (no-call) rates were assessed for the cohort, and women with low and high previous risks for aneuploidy were compared. An updated cell-free DNA algorithm blinded to the pregnancy outcome was also assessed.

Results: A total of 20,194 women were enrolled at a median gestational age of 12.6 weeks (interquartile range, 11.6-13.9). The genetic outcomes were confirmed in 17,851 cases (88.4%): 13,043 (73.1%) low-risk and 4808 (26.9%) high-risk cases for aneuploidy. Overall, 133 trisomies were diagnosed (100 trisomy 21; 18 trisomy 18; 15 trisomy 13). The cell-free DNA screen positive rate was lower in the low-risk vs the high-risk group (0.27% vs 2.2%; P<.0001). The sensitivity and specificity were similar between the groups. The positive predictive value for the low- and high-risk groups was 85.7% vs 97.5%; P=.058 for trisomy 21; 50.0% vs 81.3%; P=.283 for trisomy 18; and 62.5% vs 83.3; P=.58 for trisomy 13, respectively. Overall, 602 (3.4%) patients had no-call result after the first draw and 287 (1.61%) after including cases with a second draw. The trisomy rate was higher in the 287 cases with no-call results than patients with a result on a first draw (2.8% vs 0.7%; P=.001). The updated algorithm showed similar sensitivity and specificity to the study algorithm with a lower no-call rate.

Conclusion: In women at a low risk for aneuploidy,
single-nucleotide-polymorphism-based cell-free DNA has high sensitivity and specificity, positive predictive value of 85.7% for trisomy 21 and 74.3% for the 3 common trisomies. Patients who receive a no-call result are at an increased risk of aneuploidy and require additional investigation.

2. Norton, M. E., Jacobsson, B., Swamy, G. K., Laurent, L. C., Ranzini, A. C., Brar, H., Tomlinson, M. W., Pereira, L., Spitz, J. L., Hollemon, D., Cuckle, H., Musci, T. J., & Wapner, R. J. (2015). Cell-free DNA analysis for noninvasive examination of trisomy. *The New England journal of medicine*, *372*(17), 1589–1597. https://doi.org/10.1056/NEJMoa1407349
https://pubmed.ncbi.nlm.nih.gov/25830321/

Abstract:
Background: Cell-free DNA (cfDNA) testing for fetal trisomy is highly effective among high-risk women. However, there have been few direct, well-powered studies comparing cfDNA testing with standard screening during the first trimester in routine prenatal populations.
Methods: In this prospective, multicenter, blinded study conducted at 35 international centers, we assigned pregnant women presenting for aneuploidy screening at 10 to 14

weeks of gestation to undergo both standard screening (with measurement of nuchal translucency and biochemical analytes) and cfDNA testing. Participants received the results of standard screening; the results of cfDNA testing were blinded. Determination of the birth outcome was based on diagnostic genetic testing or newborn examination. The primary outcome was the area under the receiver-operating-characteristic curve (AUC) for trisomy 21 (Down's syndrome) with cfDNA testing versus standard screening. We also evaluated cfDNA testing and standard screening to assess the risk of trisomies 18 and 13.
Results: Of 18,955 women who were enrolled, results from 15,841 were available for analysis. The mean maternal age was 30.7 years, and the mean gestational age at testing was 12.5 weeks. The AUC for trisomy 21 was 0.999 for cfDNA testing and 0.958 for standard screening (P=0.001). Trisomy 21 was detected in 38 of 38 women (100%; 95% confidence interval [CI], 90.7 to 100) in the cfDNA-testing group, as compared with 30 of 38 women (78.9%; 95% CI, 62.7 to 90.4) in the standard-screening group (P=0.008). False positive rates were 0.06% (95% CI, 0.03 to 0.11) in the cfDNA group and 5.4% (95% CI, 5.1 to 5.8) in the standard-screening group (P<0.001). The positive predictive value for cfDNA testing was 80.9% (95% CI, 66.7 to 90.9), as compared with 3.4% (95% CI, 2.3 to 4.8) for standard screening (P<0.001).
Conclusions: In this large, routine prenatal-screening population, cfDNA testing for trisomy 21 had higher sensitivity, a lower false positive rate, and higher positive predictive value than did standard screening with the measurement of nuchal translucency and biochemical analytes.

3. Rose, N. C., Barrie, E. S., Malinowski, J., Jenkins, G. P., McClain, M. R., LaGrave, D., Leung, M. L., & ACMG Professional Practice and Guidelines Committee. Electronic address: documents@acmg.net (2022). Systematic evidence-based review: The application of noninvasive prenatal screening using cell-free DNA in general-risk pregnancies. *Genetics in medicine : official journal of the American College of Medical Genetics*, *24*(7), 1379–1391. https://doi.org/10.1016/j.gim.2022.03.019 https://pubmed.ncbi.nlm.nih.gov/35608568/

   Abstract:

   Purpose: Noninvasive prenatal screening (NIPS) using cell-free DNA has been assimilated into prenatal care. Prior studies examined clinical validity and technical performance in high-risk populations. This systematic evidence review evaluates NIPS performance in a general-risk population.

   Methods: Medline (PubMed) and Embase were used to identify studies examining detection of Down syndrome (T21), trisomy 18 (T18), trisomy 13 (T13), sex chromosome aneuploidies, rare autosomal trisomies, copy number variants, and maternal conditions, as well as studies assessing the psychological impact of NIPS and the rate of subsequent diagnostic testing. Random-effects meta-analyses were used to calculate pooled estimates of NIPS performance (P < .05). Heterogeneity was investigated through subgroup analyses. Risk of bias was assessed.

   Results: A total of 87 studies met inclusion criteria. Diagnostic odds ratios were significant (P < .0001) for T21, T18, and T13 for singleton and twin pregnancies. NIPS was accurate (≥99.78%) in detecting sex chromosome aneuploidies. Performance for

rare autosomal trisomies and copy number variants was variable. Use of NIPS reduced diagnostic tests by 31% to 79%. Conclusions regarding psychosocial outcomes could not be drawn owing to lack of data. Identification of maternal conditions was rare.

Conclusion: NIPS is a highly accurate screening method for T21, T18, and T13 in both singleton and twin pregnancies.

4. Guy, C., Haji-Sheikhi, F., Rowland, C. M., Anderson, B., Owen, R., Lacbawan, F. L., & Alagia, D. P. (2019). Prenatal cell-free DNA screening for fetal aneuploidy in pregnant women at average or high risk: Results from a large US clinical laboratory. *Molecular genetics & genomic medicine*, *7*(3), e545. https://doi.org/10.1002/mgg3.545 https://pubmed.ncbi.nlm.nih.gov/30706702/

Abstract:
Background: We evaluated the performance of a cell-free DNA (cfDNA) prenatal screening assay for trisomies 21, 18, and 13, and sex chromosome aneuploidies (SCAs) among a population of pregnant women that included both those at average and high risk.
Methods: Specimen collection, cfDNA extraction, massively parallel sequencing, and bioinformatics analysis were conducted per laboratory protocol. Assay results, concordance with pregnancy outcomes, and performance characteristics were evaluated.
Results: A total 75,658 specimens from 72,176 individual pregnant women were received. Technical reasons accounted for 288 (0.4% of all received samples) tests not performed. In the final analysis cohort (N = 69,794), 13% of pregnancies were considered at average risk and 87% at high risk. Mean gestational age at specimen collection was 15.1 weeks. Of the 69,794 unique pregnancies, 1,359 (1.9%) had positive test results. Among the results with confirmed outcomes, PPV for trisomies 21, 18, and 13 was 98.1%, 88.2%, and 59.3%, respectively; the PPV was 69.0% for SCAs and 75.0% for microdeletions. Overall, PPV was 87.2%, sensitivity was 97.9%, and specificity was 99.9%.
Conclusion: This cfDNA prenatal screening assay provides highly accurate discrimination between affected and unaffected pregnancies among a population of pregnant women at average and high risk for fetal genetic abnormalities.
Keywords: cfDNA prenatal screening assay; fetal aneuploidy; genetic counseling; microdeletion; microduplication; positive predictive value; sex chromosome aneuploidy; trisomy 13; trisomy 18; trisomy 21.

Summary of the Evidence:

Authors, date	Level of Evidence	Sample/setting	Outcomes Studies	Key Findings	Limitations/Biases
Dar et al., 2022	Large prospective multicenter cohort study	Approximately 20,000 pregnancies from multiple U.S. and international prenatal centers	Diagnostic performance of cfDNA for trisomies 21, 18, 13 Sensitivity,	cfDNA demonstrated extremely high sensitivity for trisomy 21 (~99%) and very low	Heterogeneous study population across sites Not all participants underwent identical

		Included both low-risk and high-risk populations All cfDNA results confirmed with diagnostic genetic testing or clinical follow-up	specificity, PPV, NPV Comparison with conventional screening pathways where available	false-positive rates (<0.5%) Performance remained consistent across risk groups, including low-risk first-trimester populations Fewer pregnancies were referred for invasive diagnostic testing compared to traditional screening pathways	first-trimester combined screening for direct head-to-head comparison Potential selection bias due to inclusion criteria at participating centers cfDNA platforms may vary slightly across sites
Norton et al., 2015	Prospective multicenter diagnostic accuracy trial; landmark study	18,955 pregnant women enrolled at 10–14 weeks gestation across multiple U.S. and international academic centers Included both high- and average-risk pregnancies	Direct comparison of cfDNA vs first-trimester combined screening (nuchal translucency + serum markers) Detection rate, false-positive rate, area under curve (AUC) for trisomy 21, 18, 13	cfDNA showed near-perfect diagnostic accuracy for trisomy 21 (AUC ~0.999), significantly outperforming first-trimester combined screening (AUC ~0.95–0.96) cfDNA had markedly higher specificity, resulting in substantially fewer false-positive results and fewer referrals for invasive diagnostic procedures	Study conducted in earlier cfDNA technology era (less refined than current assays) Multinational population limits strict U.S.-only generalizability Some loss to follow-up in outcome confirmation First-trimester screening protocols varied slightly between centers
Rose et al., 2022	Systematic evidence-based review; guideline-level synthesis,	Systematic review of multiple studies evaluating cfDNA in general-risk and high-risk pregnancies	Clinical utility of cfDNA in general-risk pregnancies Sensitivity, specificity, PPV,	Across pooled studies, cfDNA consistently demonstrated higher detection rates (>99% for trisomy 21 in many	Heterogeneity in included study designs and cfDNA platforms Publication bias toward positive

	ACMG-affiliated	Includes large international datasets with substantial U.S. representation	NPV Comparison with traditional serum + NT screening strategies	datasets) and significantly lower false-positive rates compared to first-trimester combined screening Review supports cfDNA as the most accurate screening tool for common aneuploidies in average-risk populations	performance studies Not a single primary dataset; indirect comparison across studies rather than uniform head-to-head analysis
Guy et al., 2019	Large retrospective real-world U.S. clinical laboratory cohort study	Large U.S. dataset from Quest Diagnostics Includes tens of thousands of pregnancies from routine clinical cfDNA screening (average-risk and high-risk patients)	Real-world performance of cfDNA screening for fetal aneuploidy Sensitivity, specificity, no-call rate, PPV Clinical utility in routine prenatal care	cfDNA demonstrated high sensitivity (~99%) for trisomy 21 and very low false-positive rates in real-world clinical use Study confirmed strong performance outside controlled trial settings and supported cfDNA reliability in routine U.S. prenatal screening practice	Retrospective laboratory-based design limits control over clinical variables Lack of standardized first-trimester combined screening comparison within same cohort Potential referral bias (higher-risk patients more likely to undergo follow-up testing) Limited clinical context beyond laboratory results

Conclusions:

Dar et al. concluded that cell-free DNA (cfDNA) screening demonstrates high diagnostic performance for trisomies 21, 18, and 13 in both low- and high-risk pregnancies, with very high sensitivity and low false-positive rates compared to traditional screening approaches.

Norton et al. concluded that cfDNA screening has significantly higher sensitivity and specificity for detecting fetal aneuploidies compared to first-trimester combined screening (nuchal translucency and serum markers), resulting in fewer false-positive results and reduced need for invasive diagnostic testing.

Rose et al. concluded that across multiple studies in general-risk pregnancies, cfDNA screening consistently outperforms traditional first-trimester screening methods in detection rates and false-positive reduction, supporting its use as a superior screening strategy.

Guy et al. concluded that in real-world U.S. clinical practice, cfDNA screening maintains high sensitivity and very low false-positive rates for fetal aneuploidy, supporting its reliability and effectiveness in routine prenatal screening across average- and high-risk populations.

Based on these four studies, cfDNA (NIPT) screening demonstrates superior diagnostic performance compared to first-trimester combined screening for detection of fetal aneuploidies, particularly trisomy 21. Across both controlled trials and real-world U.S. data, cfDNA consistently shows higher sensitivity and specificity and significantly lower false-positive rates than traditional screening methods.

Weight of Evidence

I will weigh my studies in the following order: Norton et al., Dar et al., Rose et al., and lastly Guy et al.

I weighed the Norton et al. study highest because it is a landmark prospective multicenter diagnostic accuracy trial directly comparing cfDNA with first-trimester combined screening. It provides strong head-to-head evidence showing superior sensitivity and specificity of cfDNA. A limitation is that it was conducted earlier in the development of cfDNA technology, meaning newer assays may perform even better today, and the population included both U.S. and international centers, which may slightly limit generalizability.

I weighed the Dar et al. study second because it is a large multicenter cohort study that includes both low- and high-risk pregnancies with genetic confirmation of results. I liked that it reflects more modern cfDNA performance and includes a broad population. A limitation is that not all participants had identical first-trimester screening comparisons, which limits direct head-to-head comparison strength.

Next, I weighed the Rose et al. article third because it is a systematic evidence-based review summarizing multiple studies in general-risk pregnancies. I appreciated that it supports consistent findings across the literature and provides guideline-level interpretation. However, a limitation is heterogeneity among included studies and indirect comparison rather than uniform study design.

I weighed the Guy et al. study last because, while it provides strong real-world U.S. clinical laboratory data, it is retrospective in nature and does not directly compare cfDNA to first-trimester combined screening within the same cohort. However, it is valuable for confirming that cfDNA maintains high performance in routine clinical practice outside of controlled trial settings.

Clinical Bottom Line:

Based on these four studies, cfDNA (NIPT) screening demonstrates superior diagnostic performance compared to first-trimester combined screening for detection of fetal aneuploidies, particularly trisomy 21. Across both controlled trials and real-world U.S. data, cfDNA consistently shows higher sensitivity and specificity and significantly lower false-positive rates than traditional screening methods.

Magnitude of any effects:

The magnitude of effect is high, as cfDNA consistently demonstrates significantly improved sensitivity and specificity compared to first-trimester combined screening, with a marked reduction in false-positive rates across all included studies.

Clinical Significance

Clinically, these findings support cfDNA as the preferred screening modality for fetal aneuploidy in first-trimester prenatal care. The reduction in false-positive results is particularly important, as it decreases unnecessary anxiety and reduces the need for invasive diagnostic procedures such as chorionic villus sampling or amniocentesis. Given comparable or superior performance across diverse populations, cfDNA should be offered as the primary screening option in appropriate patients, with shared decision-making based on patient values and access.

Other Considerations

Future studies should continue to evaluate cfDNA performance in diverse populations and ensure equitable access across healthcare systems. Additionally, cost-effectiveness and implementation studies are important, as cfDNA screening may not yet be universally available or covered in all settings. Further head-to-head comparisons using contemporary cfDNA platforms would also strengthen the evidence base, particularly in low-risk populations where traditional screening is still widely used.