

# Diagnostic Accuracy of Noninvasive Detection of Fetal Trisomy 21 in Maternal Blood: A Systematic Review

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## Key Words

Noninvasive prenatal diagnosis · Screening · Trisomy 21 · Down syndrome · Maternal blood

## Abstract

**Background:** Both pregnant women and providers of obstetric care are aware of the rapid advances in noninvasive prenatal diagnosis (NIPD) of fetal trisomies, and appear to look forward to its clinical introduction. **Objectives:** To review and critically assess the published literature on diagnostic accuracy of NIPD using cell-free fetal DNA or RNA in maternal blood to detect fetal trisomy 21. **Method:** An electronic search was performed in MEDLINE, EMBASE and the Cochrane library (1997 to April 2011). Of a total of 201 citations, 9 studies were eligible for full-text analysis by 2 independent reviewers, using the QUADAS tool. **Results:** Two of the 9 analyzed studies complied with the criteria of the QUADAS tool. Combining the selected 2 studies, with a total of 681 pregnancies included, overall sensitivity was 125/125 (100%, 95% CI 97.5–100%) and specificity 552/556 (99.3%, 95% CI 98.7–99.3%). **Conclusions:** NIPD of fetal trisomy 21, using fetal nucleic acids in maternal plasma, appears to have a high diagnostic accuracy. Large-scale prospective studies are awaited before implementation in clinical practice.

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## Introduction

Pregnant women have been offered prenatal diagnosis to detect trisomy 21 (T21, Down syndrome) since the 1970s. T21 is the most common chromosomal abnormality with a birth prevalence of 11–14 per 10,000 [1–3]. In most countries, only women considered to be at relatively high risk of a chromosomally abnormal fetus are offered diagnostic testing, since the only available methods are chorionic villus sampling or amniocentesis, invasive procedures with inherent hazards to the pregnancy. Selection of a high-risk group was for many years done based only on maternal age, with low detection rates and hundreds of invasive tests needed to find 1 fetus with T21. Screening has improved by using a combination of maternal serum markers and nuchal translucency measurement, the combination test, in the best programs identifying 90% of T21 cases for 5% false positives [4].

Since the first report in 1997 by Lo et al. [5] of the possibility to use circulating cell-free fetal DNA in maternal plasma for fetal diagnosis, there has been the expectation that some day the complex multimarker, operator-dependent screening test and the invasive testing for karyotyping could be replaced by just taking a maternal blood sample. In the past few years, determination of fetal sex and Rh-D type using cell-free fetal DNA in maternal

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plasma already has become routine clinical practice [6–9]. Detecting fetal T21 however is technically more challenging, since maternal plasma contains both cell-free fetal and maternal DNA fragments. Various methods to diagnose fetal trisomy using maternal plasma DNA or RNA have been developed. Recently, several relatively small studies have been published reporting on the diagnostic accuracy of these methods.

Most authors of these studies agree that there is a need to evaluate the performance of these new tests in large unselected populations. In order to prepare for such studies, we aimed to systematically review and critically appraise the published literature, using the QUADAS guidelines [10], on the accuracy of noninvasive methods using cfDNA or mRNA from maternal plasma to detect fetal T21.

## Methods

This systematic review was conducted using a protocol with generally accepted methods [11].

### *Eligibility Criteria*

We considered all studies from 1997 until May 2011 in which diagnostic accuracy was determined for noninvasive detection of T21 using nucleic acids, DNA or mRNA, in maternal plasma regardless of the method used. For a study to be included in our review, the noninvasive detection method had to be compared to the gold standard for the determination of trisomies, karyotyping or rapid aneuploidy detection using fluorescent in situ hybridization, quantitative fluorescent polymerase chain reaction or multiplex ligation-dependent probe amplification on fetal, placental or neonatal cells. Evaluation of the quality of the studies was done using the QUADAS tool.

### *Information Sources and Search*

Librarians from the Walaeus Library, University of Leiden, searched MEDLINE, EMBASE and the Cochrane Library for relevant papers. The Medical Subject Headings (MeSH) terms ‘prenatal (diagnosis)’, ‘Down syndrome’, ‘aneuploidy’ were used, and combined by Boolean operators (‘and’ and ‘or’) with ‘noninvasive’, ‘non-invasive’ and ‘maternal’. In addition, the reference lists of all primary articles and recent articles, editorials and reviews published on noninvasive prenatal diagnosis (NIPD) were screened to identify articles not found by the initial search. No restrictions were used for publication type or language.

### *Study Selection*

Two trained reviewers independently screened titles and abstracts for relevance (E.J.V. and M.A.d.B.). Selected full papers were independently evaluated for inclusion and analysis (E.J.V. and D.O.). Studies were independently assessed by 2 reviewers (E.J.V., M.A.d.B.) for methodological quality against the quality assessment of diagnostic accuracy studies (QUADAS) criteria [10]. Disagreements were resolved by consensus including a third

reviewer (D.O.). In case of multiple publications of one dataset we included only the most recently published study.

### *QUADAS*

The QUADAS criteria are a validated evidence-based tool consisting of a 14-item checklist which encompasses the most sources of bias and variation observed in diagnostic accuracy. The quality assessment items are: representative patient spectrum, description of selection criteria and reference standard, acceptable interval before outcome, partial and differential verification, incorporation bias, adequate test description, blinding of index and reference test, clinical data available and description of uninterpretable test results. All reviewers were trained using the QUADAS tool. Each item was scored ‘yes’, ‘no’ or ‘unknown’ as recommended by the authors of the QUADAS tool [10].

### *Data Extraction*

We included test accuracy studies allowing construction of one or more  $2 \times 2$  contingency tables for each study containing the various methods of noninvasive detection of T21 cross-classifying with the gold standard. We combined results from all selected studies to assess an overall sensitivity and specificity of NIPD to detect T21, with 95% confidence intervals (CI).

## Results

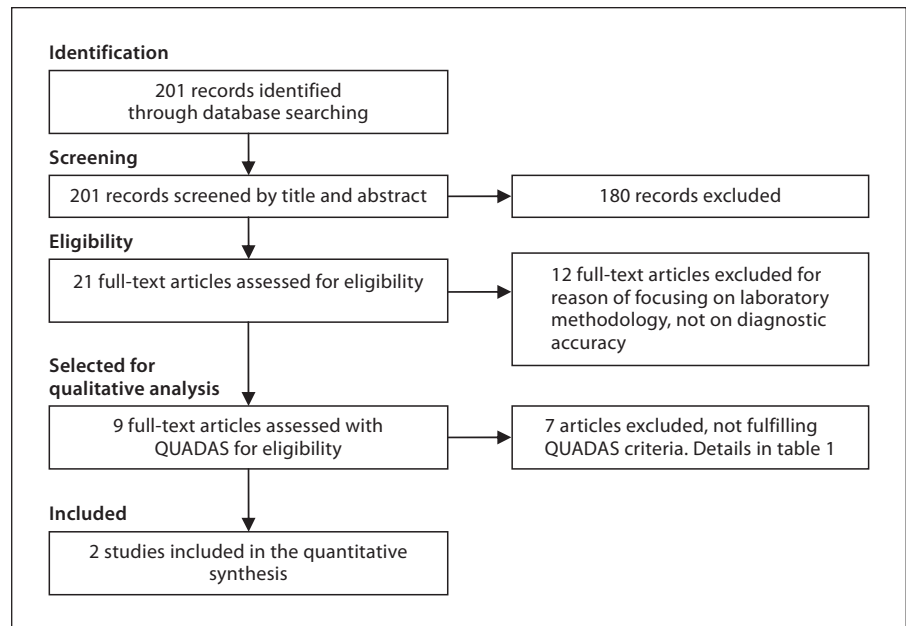
### *Included Studies*

Figure 1 summarizes the selection process. From the initial 201 publications, 21 full-text articles remained after evaluation of title and abstract. Another 12 studies were excluded after reading the full text focusing on methodology of the laboratory process rather than the performance of the test. The remaining 9 studies were assessed for eligibility and discussed by the expert panel. In case of unknown information we contacted the authors for more information. All studies were scored using the QUADAS instrument [10].

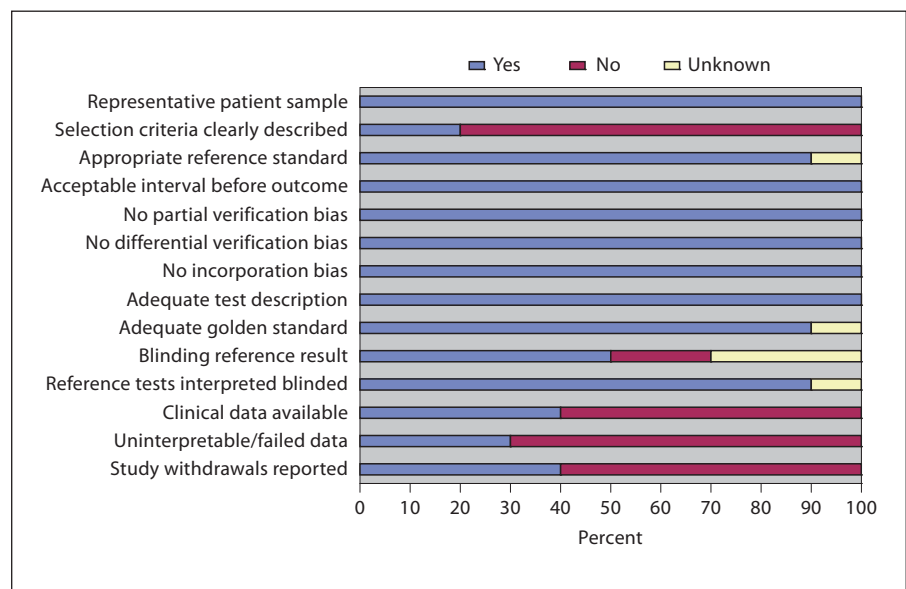
Seven studies [12–18] failed to meet the required criteria for diagnostic test evaluation according to the QUADAS instrument (table 1; fig. 2).

In all these studies sampling was performed only in high-risk pregnancies with an indication for invasive testing and compared with the golden standard of karyotyping. The test description was adequate in all studies. The 9 studies used different methods of noninvasive testing as well as different calculation methods and different cutoffs for standardized fractional genomic presentation (Z score).

After scoring all studies following the QUADAS criteria, 2 studies remained for quantitative synthesis. The study with the largest sample size [19] investigated 753 samples of pregnant women with a high risk of fetal T21 with 2 test methods, one 8-plex and one 2-plex procedure.



**Fig. 1.** Flow through the different phases of the systematic review.



**Fig. 2.** Summary of quality of the reviewed studies using the QUADAS instrument.

The 2-plex showed the best performance, used in a total of 232 samples of which 86 were from T21 cases. The inclusion criteria were singleton pregnancies with clinical indications for chorionic villus samples or amniocentesis. The investigators used both prospectively recruited samples as well as archived maternal samples. The sensitivity was 86/86 (100%) and the specificity was 143/146 (97.9%). The median gestational age at the time of maternal blood sampling was 13 weeks and 1 day. Insufficient quality of

samples was present in 5.6%. Failure to obtain results occurred in 1.5%.

The study by Ehrich et al. [20] prospectively tested 480 high-risk pregnancies with 39 women carrying a T21 fetus. They used a multiplexed massively parallel shotgun sequencing assay. High-risk pregnancies were described as pregnancies with clinical indications for chorionic villus sampling or amniocenteses including a positive combination test, maternal age >35 years, family history with

**Table 1.** Overview of excluded studies that were assessed using the QUADAS instrument

Reference	n	n T21	Sensitivity %	Specificity %	Method	Sampling	Comments according to QUADAS criteria
Fan et al. [12], 2008	18	9	100	100	Massive parallel genomic sequencing	After invasive procedure	Selection criteria not clearly described, selected samples used, failed or retested samples not mentioned
Chiu et al. [13], 2008	28	14	100	100	Massive parallel genomic sequencing	Euploid samples before invasive procedures and T21 samples before and after invasive procedure before TOP	Selected samples used, failed or retested samples not mentioned
Tong et al. [14], 2010	24	5	100	95.8	Epigenetic-genetic chromosome-dosage approach	Not mentioned	Selection criteria not clearly described, no blinded samples, failed samples not mentioned
Tsui et al. [15], 2010	153	16	100	89.7	PLAC4 SNP (RNA) by mass spectrometric and digital PCR methods	Before invasive procedure	Failed samples not mentioned, clinical data not clearly described
Ghanta et al. [16], 2010	40	7	100	100	Tandem SNP	Before and after invasive procedure	Selection criteria not clearly described, selected samples used
Deng et al. [17], 2011	121	23	92	100	PLAC4 SNP (RNA) RT-MLPA	Before invasive procedure	Selection criteria not clearly described, failed samples not mentioned
Papageorgiou et al. [18], 2011	40	14	100	100	Methylated DNA immunoprecipitation	Archived samples, time of sampling not mentioned	Selection criteria not clearly described, archived samples used, failed samples not mentioned

n T21 = Number of women carrying a trisomy 21 fetus; TOP = termination of pregnancy; SNP = single-nucleotide polymorphisms; RT-MLPA = reverse transcriptase multiplex ligation-dependent probe amplification.

T21, a previous T21 pregnancy or ultrasound abnormalities suggestive of T21.

The samples were collected prospectively, but were analyzed later (all within 10 months after sampling). None of the samples were analyzed prospectively or as fresh samples.

The sensitivity was 100% and the specificity was 99.7%. One sample was misclassified as T21 (false positive). The median gestational age at blood sampling was 16 (range 8–36) weeks. Ehrich et al. [20] described a sample loss of 2.6% before processing for several reasons (plasma volume <3.5 ml; one sample tube dropped during DNA ex-

traction; samples mixed into each other, and tube broken during centrifugation). In 3.8% the sample was excluded during the process for a variety of reasons including insufficient percentage of fetal DNA, total DNA or library concentration. Three of the samples excluded from analysis were identified as T21.

Overall, combining the results by Ehrich et al. [20] and the 2-plex data from the study by Chiu et al. [19], 681 samples with 125 T21 cases analyzed by massively multiplexed parallel sequencing resulted in a sensitivity of 100% (95% CI 97.2–100) and a specificity of 99.3% (95% CI 98.7–99.3; table 2).

**Table 2.** Overview of the included studies

First author, year	n	n T21	Sensitivity %	Specificity %	Method
Chui et al. [19], 2011					
8-plex	753	86	79.1	98.9	Massive parallel genomic sequencing
2-plex	232	86	100	97.9	
Ehrich et al. [20], 2011	480	39	100	99.7	Massive parallel genomic sequencing
Combined data Ehrich and Chiu 2-plex protocol	681	125	100	99.3	

## Discussion

### *Summary of Evidence*

From the recent literature, we can conclude that, after more than a decade of research, NIPD of fetal T21 has become a clinical reality. The 2 studies included meeting all items of the QUADAS criteria aimed to validate the multiplexed massively parallel sequencing. Both studies suggest that T21 can reliably be detected early in the first trimester from maternal plasma with a sensitivity and specificity of nearly 100%. Yet in a number of samples (1.5–3.8%) no results could be obtained. From these excellent results however, we cannot conclude that this new test will have similar performance when implemented into routine obstetric care. The current evidence seems to almost justify such use, however, in the studies performed the samples were mostly stored and then run in a large batch, while for true clinical use, a real-time rapid testing for each patient is needed. This was acknowledged by the authors, who stated that their promising method requires clinical validation in a larger multicenter study. As Chiu et al. [19] concluded, more research is needed to evaluate the use of NIPD as a first-line test for all pregnant women. All studies published thus far were based on high-risk pregnancy samples.

The sensitivity of the current type of screening for the detection of T21 in clinical practice, the combination test, varies from 70 to 91%. The false-positive rate is usually set at 5%, with cutoff values around 1:200 to separate high risk from normal risk. This screening policy, reporting of risks and counseling of pregnant women, is considered by most to be complex and time-consuming. Uptake of screening varies enormously per country, with <30% in the Netherlands to >90% in Denmark. The false-positive rate results in many invasive tests in healthy pregnancies, with one procedure-related miscarriage of a healthy fetus for every 2–3 T21 detected [21–24].

The method used for noninvasive trisomy detection in the studies by Ehrich et al. [20] and Chiu et al. [19] was massive parallel sequencing of maternal plasma DNA. Other studies however used a variety of alternative methods, including tandem single-nucleotide polymorphism array, RNA to single-nucleotide polymorphism allelic ratio approach, reverse transcriptase multiplex ligation-dependent probe amplification, epigenetic-genetic chromosome dosage studies and others. Rapid improvements in these methods, the platforms used and in data analysis, leading to even more reliable, faster and cheaper testing, are expected in the near future. Which method will be preferable for clinical use remains to be elucidated.

It is still unclear whether evaluation for fetal trisomy using maternal plasma nucleic acids is feasible and reliable in the first trimester. Published studies have used blood samples from a wide range of gestational ages, with insufficient numbers to assess accuracy per week gestation. Other important aspects that require further study are failure rate, need for retest rate, and time to reporting to the patient.

A next step will be a large prospective study in a low-risk population in a real-life setting in which apart from test characteristics throughput capacity, turnaround times, and costs need to be studied. The costs will need to be weighted against the costs of the current practice of performing a combination test and invasive procedures.

### *Limitations*

The main limitation of using the QUADAS tool to evaluate selected studies is that it relies on published data. Some studies may receive a negative score on certain items based on unclear reporting, while the study itself may have met the criteria. We have tried to overcome this by contacting the investigators for more detailed information. Not all authors responded to these requests. Received responses were imported into our results.



## Conclusion

Both pregnant women and providers of obstetric care are aware of the rapid advances in NIPD, and appear to look forward to its clinical introduction. Therefore, there is some urgency to perform large-scale properly conducted clinical evaluation studies while we still can. Consumer-driven genetic testing and commercial parties offering tests to anyone who pays may interfere with

scientific and diagnostic evaluation. We believe now is the time, preferably in multicenter and if needed international collaboration, to design and carry out large-scale studies to rigorously analyze the diagnostic accuracy and cost effectiveness of NIPD. In parallel, we should also thoroughly evaluate all ethical and social implications of the revolutionary changes in prenatal diagnosis that await us.

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