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Review Article

# Noninvasive prenatal diagnosis

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ABSTRACT

Prenatal examination plays an important role in present medical diagnosis. It provides information on fetal health status as well as the diagnosis of fetal treatment feasibility. The diagnosis can provide peace of mind for the perspective mother. Timely pregnancy termination diagnosis can also be determined if required. Amniocentesis and chorionic villus sampling are two widely used invasive prenatal diagnostic procedures. To obtain complete fetal genetic information and avoid endangering the fetus, noninvasive prenatal diagnosis has become the vital goal of prenatal diagnosis. However, the development of a high-efficiency separation technology is required to obtain the scarce fetal cells from maternal circulation. In recent years, the rapid development of microfluidic systems has provided an effective method for fetal cell separation. Advantages such as rapid analysis of small samples, low cost, and various designs, greatly enhance the efficiency and convenience of using microfluidic systems for cell separation. In addition, microfluidic disks can be fully automated for high throughput of rare cell selection from blood samples. Therefore, the development of microfluidic applications in noninvasive prenatal diagnosis is unlimited.


## Introduction

Prenatal diagnosis is an important medical technology for nearly two centuries. The procedure can notify parents of hereditary diseases in the fetus such as Down syndrome, sickle cell anemia, Edwards's syndrome, cystic fibrosis, and Duchenne muscular dystrophy. These diseases may cause neonate stunted growth, intellectual disability, physical disability, and death. To date, expecting parents can select various methods to confirm fetal health. However, different diagnosis methods may be presented with different levels of risks, where invasive prenatal diagnosis procedures may induce miscarriage risks. Therefore, the development of safe and highly valuable prenatal diagnostic techniques is greatly sought after for scientists around the world.

The first prenatal diagnosis can be traced back to as early as the 20th century, after Wilhelm Röntgen's [1] discovery of X-rays. Although X-rays can be utilized to observe fetus appearance, it provides no genetic diagnosis. In 1966, Steele and Breg [2] separated fetal chromosomes from amniotic fluid for chromosome analysis which has laid the foundation of amniocentesis in modern medicine. Later, an Italian biologist, Simoni et al [3], performed the first trimester chorionic villus sampling (CVS) and risk assessment to validate this method as a reliable prenatal diagnosis tool [3,4]. These two methods are regarded as a model for prenatal diagnosis; however, their invasiveness may lead to a risk of miscarriage. Due to the potential risks of invasive prenatal genetic diagnosis, different noninvasive prenatal diagnosis (NIPD) techniques are actively being developed.

## Invasive prenatal diagnosis

Although invasive prenatal diagnosis is dangerous, there exists no effective replacement among today's technologies. Table 1 indicates the most direct methods of siphoning fetal samples from the mother and their associated risk of miscarriage [5–9].

### *Amniocentesis*

Amniocentesis was used to treat pregnant women with polyhydramnios in 1880. In 1966, American physicians Steele and Breg

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**Table 1**
Comparison of invasive prenatal diagnosis.

| | Execution time (wk) | Sampling location | Diagnosis | Risk of miscarriage (%) | Refs |
|---|---|---|---|---|---|
| Chorionic villous sampling | 10–12 | Chorionic villus | Chromosomal abnormalities | 0.5–2 | [5] |
| Amniocentesis | 14–16 | Amniotic sac | Chromosomal abnormalities/neural tube defects | 0.06–1.3 | [6,7] |
| Fetal blood sampling | ≥17 | Fetal umbilical cord | Chromosomal abnormalities/metabolic disorders/fetal infections | 2–3 | [8,9] |

FISH = fluorescence in situ hybridization; PCR = polymerase chain reaction.

[2] successfully cultured amniotic fluid cells for chromosome analysis. Presently, it is the most widely used invasive prenatal diagnosis because of its high accuracy and low risk characteristics. Previous research has suggested that spontaneous abortion percentage after amniocentesis is 1.7% [10], which was slightly higher than the control group that underwent only ultrasonic examinations. The report in 2000 also suggested that the fetus miscarriage risk of amniocentesis is ~0.6–0.68% [11]. This method is applicable for women at 16–18 weeks of pregnancy, and ~200–300 mL of amniotic fluid in the uterus of pregnant women is required. Amniotic fluid contains ~2–3 $\times$ $10^5$ cells per 10 mL, and these cells are produced by fetal movements in the amniotic sac due to swallowing, urination, and physical movements [12]. Amniocentesis is useful for the diagnoses of many single-gene diseases and congenital defects such as Down syndrome, thalassemia, Adrenoleukodystrophy, and Huntington's Disease.

*Chorionic villous sampling*

The advantage of CVS is that it is suitable for early screening for women at around 10–13 weeks of pregnancy and in special cases it can be performed as early as 8 weeks [13]. This approach extracts tissue and fetal placental chorionic cells transcervically or transabdominally, where the transabdominal method has been confirmed to be safer than the transcervical method in a previous report [14]. The miscarriage risk of CVS is higher than amniocentesis, which is ~0.5–2%. The fetal diseases that can be identified by CVS are similar to those of the amniocentesis, which include chromosomal abnormalities and genetic defects [5].

*Percutaneous umbilical cord blood sampling*

Cordocentesis, also known as percutaneous umbilical cord blood sampling (PUBS), draws blood samples directly from the fetal umbilical vein with a sampling needle. This method is suitable for women at the second trimester pregnancy stage, generally performed after 17 weeks of pregnancy [15]. The late sampling time of PUB than amniocentesis and CVS is because the early fetal umbilical vein is fragile and not suitable for puncture. Studies that investigated umbilical cord puncture procedure and fetal damage found no obvious pathological symptoms a week after PUBS [16]. Such direct sampling method has certain risks associated with gestational age, operating procedures, and sampling frequency of needle piercing, with different outcomes [8]. A statistical analysis has indicated that PUBS causes ~2–3% of miscarriages [9]. However, PUBS can be used to diagnose fetal chromosomal abnormalities, infections, and metabolic disorders that cannot be determined with CVS or amniocentesis.

## NIPT

Currently, many scientists have been developing NIPT techniques for collecting fetal samples in order to reduce the risk of miscarriage from invasive procedures. Table 2 summarizes each technique and its disadvantages [17–28].

*Ultrasonography*

Ultrasonography is generally considered as a safe method of image rendering. It is one of the most common NIPT technologies, where images are formed when the echoes of ultrasound that penetrated the uterus tissue is received. The sound waves are reflected to the receiving probe with various interfaces, and after concussion, the signals are transformed to electrical signals to render two-dimensional, three-dimensional, or higher images. Ultrasound is often used for examining fetal congenital disability or abnormal development, by observing fetus nuchal translucency and nose bone development, which can predict the possibility of a fetus suffering from Down syndrome. However, in clinical practice, Down syndrome diagnosed with ultrasonography has a high false positives rate of ~5% [17]. Although ultrasonography examination cannot detect most of the inherited diseases or genetic defects, it is still an indispensable NIPT tool.

*First and second trimester screening*

Second trimester maternal serum screening includes double, triple, and quadruple tests. It is one of the most commonly chosen examinations. This method determines specific protein concentrations in maternal serum to calculate the likelihood and risk

**Table 2**
Comparison of noninvasive prenatal testing.

| | Execution time (wk) | Target | Diagnosis | Detection rate for Down's syndrome (%) | Disadvantage | Ref. |
|---|---|---|---|---|---|---|
| Ultrasonography | <20 | (1) Nuchal translucency<br>(2) Nose bone | Down's syndrome<br>Deformity | 80–90 | High false positive (4.5–6.0%) | [17–19] |
| Triple test | 16 | (1) α-fetoprotein<br>(2) Oestriol<br>(3) hCG | Down's syndrome<br>Neural tube defects | 60–70 | No definitive diagnosis | [20,21] |
| Cell-free fetal DNA (mRNA) in maternal blood | >12 | (1) Fetal mRNA<br>(2) Fetal DNA | Single-gene disorders<br>Aneuploidy | >99 | Large sample<br>Cost effectiveness | [22–25] |
| Fetal cell in maternal blood | 4–14 | (1) Fetal lymphocytes<br>(2) Trophoblasts<br>(3) Nucleated red blood cells | The disease can be detected by PCR or FISH | 75 | Very rare | [26–28] |

FISH = fluorescence in situ hybridization; PCR = polymerase chain reaction.