In the broadest sense, noninvasive prenatal testing began in the 1970s, when the first articulated-arm diagnostic ultrasound machines produced two-dimensional images of fetuses in utero. Initially, the crude new imaging technology was eagerly adopted for the simple measurement of embryos, fetuses, and fetal parts to develop normative data for gestational-age determination. But as technology and image quality rapidly improved, it quickly became obvious that fetal anatomy could be defined with increasing precision.

At approximately the same time, Brock and Sutcliffe recognized that alpha-fetoprotein, which is found in very high concentrations in fetal blood and cerebrospinal fluid, could be measured in maternal serum at much lower concentrations. Elevated levels of this protein were associated with, but not perfectly predictive of, open neural-tube defects and other fetal abnormalities. Merkatz et al. recognized that unusually low serum levels of maternal alpha-fetoprotein were associated with an increased risk of fetal Down's syndrome, and the rush was on to develop other maternal serum and sonographic markers to improve the positive and negative predictive values for the prenatal screening process.

What is fundamentally different about the new era in noninvasive prenatal diagnosis of aneuploidy is that for the first time, the analyte of interest in maternal blood is DNA and not a proxy analyte associated with, but not directly responsible for, the fetal phenotype of interest. Lo et al. opened the door to the new era when they described procedures for identifying qualitatively different DNA sequences in the maternal circulation that could originate only in a fetus: Y chromosome sequences from a male fetus and DNA encoding the rhesus D antigen (so-called Rh factor) in women who were Rh-negative. Initially, these sequences were obtained by disrupting rare fetal cells in the maternal circulation, but later they were obtained from the small fragments of cell-free DNA (cfDNA) that are shed by the placenta into the maternal circulation. The proportion of fetal cfDNA circulating in maternal blood increases during gestation and represents about 10% of free DNA in circulating maternal blood during the first and second trimesters.

The most recent advances have been made possible by the development of very rapid DNA sequencing and computer hardware and software capable of matching hundreds of millions of DNA fragments and sequences in hours. Sequencing of the circulating DNA obtained from a woman carrying a euploid fetus provides random sequence reads (the nucleotide sequence of stretches of DNA) of each chromosome. Sequence reads that are derived from a specific autosomal chromosome have a 1:1 ratio with the reads of any other autosomal chromosome. Fetuses with aneuploidy have a chromosomal dosage imbalance, which can be detected through deviations from a 1:1 ratio, in the number of chromosome-specific sequence reads. However, the relatively high proportion of maternal DNA in maternal blood has posed a challenge to the sensitive detection of fetal aneuploidy.

Massive parallel sequencing, also called next-generation sequencing, generates millions of sequence reads along the length of each chromosome, thereby overcoming the challenge of excess maternal DNA and providing the sensitivity
required for the determination of fetal aneuploidy. Studies to date have shown the sensitivity and specificity of this technology for the screening of cfDNA in composed study populations with known karyotypes with very high prevalences of aneuploidies.\textsuperscript{5,6} This study method, however, has made it impossible to calculate the positive and negative predictive values of cfDNA screening at prevalences of aneuploidies that would be encountered in a generally representative obstetric population.

In this issue of the Journal, Bianchi et al.\textsuperscript{7} describe how cfDNA screening is also useful in women at low risk for carrying an aneuploid fetus. The investigators compared the performance of cfDNA screening with that of standard screening (serum biochemical assays with or without measurement of nuchal translucency) in singleton pregnancies. The primary outcome was the comparison of false positive rates for identifying trisomy 21 (Down’s syndrome) and trisomy 18 (Edwards’ syndrome). They observed that cfDNA screening had a greater specificity than the standard biochemical and imaging methods of screening in that it had a significantly lower false positive rate than standard screening (0.3\% vs. 3.6\% in detecting trisomy 21 and 0.2\% vs. 0.6\% in detecting trisomy 18). In addition, they reported a lower rate of false positives with cfDNA screening for trisomy 13 (Patau’s syndrome) than with standard screening (0.1\% vs. 0.7\%), although the difference was not statistically significant.

The positive predictive values of the assay — 45.5\% for trisomy 21 and 40.0\% for trisomy 18 — underscore the conclusion that assaying fetal DNA is a screening tool and not a diagnostic intervention. As the investigators acknowledge, women who receive a positive result on cfDNA screening must be counseled to have a diagnostic test — for example, through karyotype analysis of cells obtained by amniocentesis or chorionic villus sampling — to determine whether their fetus is one of the approximately 60\% of fetuses that are falsely identified on cfDNA screening as having a chromosome 18 or 21 trisomy.

The observed negative predictive values of 100\% with 95\% confidence limits down to 99.8\%, combined with the significantly and substantively lower false positive rates with cfDNA screening than with standard screening, augurs well for pregnant women and their fetuses: a negative result on cfDNA screening obviates the need for invasive testing and thus the discomfort and risk to the pregnancy incurred by such testing.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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**New Diagnostics for Common Childhood Infections**

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The implementation of preventive strategies and effective treatment has substantially reduced the incidence of malaria across many parts of Africa.\textsuperscript{1} The introduction of *Haemophilus influenzae* type b vaccine and, more recently, pneumococcal conjugate vaccine should dramatically reduce the incidence of serious bacterial infections among children.\textsuperscript{2} Historically, these pathogens...