with vitamin B\textsubscript{12}, but both continue to have symptoms of peripheral neuropathy. There have been reports of false normal results for vitamin B\textsubscript{12} levels generated by automated analyzers when the serum of patients with megaloblastic anemia is evaluated. The results have been attributed to the possibility that high levels of intrinsic factor–blocking antibodies interfere with the assay.\textsuperscript{1,2} Today, vitamin B\textsubscript{12} assays are primarily performed on automated analyzers that apply a method based on the competitive binding of serum vitamin B\textsubscript{12} with reagent intrinsic factor. Many of these platforms have also been found to be inaccurate when serum containing intrinsic factor–blocking antibodies is analyzed.\textsuperscript{2} Disconcertingly, pernicious anemia is the most common cause of vitamin B\textsubscript{12} deficiency, and up to 70% of patients with pernicious anemia have intrinsic factor–blocking antibodies.\textsuperscript{3}

To investigate further, we precipitated serum immunoglobulins by adding 25% polyethylene glycol (PEG) by volume in a 1:1 dilution with serum. Using unmodified and PEG-treated samples of serum from the two patients and from three controls (patients without macrocytic anemia), we then ran tests for vitamin B\textsubscript{12} levels (Fig. 1). In the PEG-treated samples from the two patients, vitamin B\textsubscript{12} levels decreased to below the limit of detection; the PEG-treated samples from the controls showed a decrease compatible with the 1:1 dilution.

We have been performing vitamin B\textsubscript{12} assays on the Siemens Dimension Vista system at our institution. A review of the package insert shows that the manufacturers are aware of this issue and recommend testing for intrinsic factor–blocking antibodies if test results are in conflict with the clinical diagnosis. We are in the midst of evaluating other platforms for this assay and have notified our clinicians of the issues described. However, we are concerned that there is insufficient awareness in the medical community of the possibility of spuriously high vitamin B\textsubscript{12} levels; we urge pathologists to review their methods and clinicians to incorporate the information presented here into their diagnostic evaluations.

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


**A Noninvasive Test to Determine Paternity in Pregnancy**

**TO THE EDITOR:** Five percent of women who are raped become pregnant, which results in an estimated 32,000 pregnancies annually in the United States.\textsuperscript{1} In many circumstances, it is unclear whether the pregnancy resulted from the rape or from consensual intercourse. The only options available for prenatal paternity determination are invasive tests, such as the sampling of chorionic...
villi and amniocentesis, that carry a risk of miscarriage and are not performed before 10 to 15 weeks of gestation. Because 78.9% of terminations of unintended pregnancies are carried out before 10 weeks, it seems likely that many rape victims terminate pregnancies before testing for paternity. A noninvasive prenatal paternity test based on cell-free fetal DNA present in maternal blood, performed at 8 weeks of gestation or later, could provide a safe option for determining paternity.

Previous studies of noninvasive prenatal paternity testing have shown that amplification of fetal alleles from maternal blood is suppressed by the presence of cell-free maternal DNA. Furthermore, fetal DNA in maternal plasma is highly degraded. These limitations can be overcome by first adding a fixative to maternal blood samples.
to stabilize cell membranes and prevent the release of maternal DNA into the plasma. By using single-nucleotide polymorphisms to distinguish fetal DNA from maternal DNA (Fig. 1), one can use short amplicons (shorter than 75 bp) to minimize allele dropout (absence of a fetal DNA signal when one should be present).

We collected blood samples from 30 women with pregnancies of 8 to 14 weeks of gestation. Each maternal blood sample was paired with blood from the biologic father and then randomly grouped with 1 of 29 samples from unrelated men. The 3 samples in each group were processed in a blinded manner. We determined paternity correctly for all 30 samples, by comparing the genetic profile of fetal DNA in maternal blood with those of the 2 “paternal” samples (1 genuine, 1 not) (Table 1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). The odds of identifying the correct father for all 30 samples are less than 1 out of 1 billion ($P = 1.86 \times 10^{-9}$). Our approach shows that noninvasive prenatal paternity testing can be performed within the first trimester with the use of a maternal blood sample.

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