RhD GENOTYPING OF THE FETUS USING AMNIOTIC FLUID

To the Editor:

We read with interest the letter by Wolter et al.1 in which they describe a polymerase chain reaction (PCR) method for RhD genotyping.

A number of indications were mentioned by Wolter et al., including fetal Rh testing using chorionic villus biopsy or fetal-derived white blood cells in the maternal circulation. We described2 the determination of the RhD status of the fetus early in pregnancy without transgressing the fetomaternal circulation. PCR was used to amplify regions of the RhCcEe and RhD genes or a segment of the RhD gene alone.3,4 Material used was DNA derived from amniocytes in 15 pregnancies with confirmatory serological RhD typing on fetal blood. In addition, RhD genotyping was performed on chorionic villus samples obtained before termination of pregnancies and the results were compared with those from PCR typing from DNA obtained directly from fetal tissue after termination. In all cases there was complete agreement between fetal RhD genotyping by PCR both from amniocentesis and chorionic villus sampling, as demonstrated by serology on fetal blood samples and PCR from isolated fetal tissue. The method of choice of RhD genotyping of the fetus in the first trimester is amniocentesis, because chorionic villus sampling is associated with fetal risk5 and with fetomaternal hemorrhage.6 Fetomaternal hemorrhage may increase the maternal alloimmunisation. However, amniocentesis in the first trimester may also be a risk and volumes obtained should be kept to the minimum.7

Amniocentesis is now used routinely by our group to RhD type the fetus at the time of first amniocentesis for maternal RhD alloimmunization to determine whether the fetus is at risk. This is applicable to fetuses whose paternity is unknown or whose father is heterozygous for the RhD antigen. It is also extremely convenient because an amniocentesis sample can be sent to a center performing PCR RhD genotyping without the need for referring all pregnant women with anti-D for fetal blood sampling. This avoids unnecessary invasive fetal blood sampling.8
With regard to RhD zygosity testing, while recognizing the importance of the clinical application of such a test, we doubt the claims that the method described by Wolter et al can be reliably used to determine RhD zygosity. PCR is only linear with respect to starting material over a limited range of cycle numbers. Our experience with the use of quantitative reverse transcriptase PCR for mRNA analysis shows that the linear range, with low concentrations of template DNA, is usually less than 30 cycles. As the concentration of template DNA increases, the number of cycles to maintain a linear relationship decreases. Because the concentration of DNA in this application is likely to be high, we suspect that 35 cycles will take the procedure out of the linear range, making zygosity determination unreliable. Further experiments will be needed to optimize the quantitative aspects of the reaction and a large number of subjects will need to be studied (through making comparisons with serology and Southern blotting) to prove its reliability.

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REFERENCES
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