RhD determinations published, mainly about fetal sex and presence of cell-free fetal DNA in maternal serum.

Since the first demonstration of the presence of cell-free fetal DNA in maternal serum during the first trimester of pregnancy was estimated to range from 5.2 to 64.9 copies/mL (mean, 26.3 copies/mL). This result was similar to those obtained by others (9). The CV of the test in this range of concentration was ±2% (one sample analyzed 12 times during one analytical run; mean cp, 38.6; SD, 0.8 cp), which reflects the reproducibility of both DNA extraction and PCR assay.

Analysis of fetal DNA in maternal serum offers new possibilities for noninvasive prenatal diagnosis. A lack of contamination is crucial for subsequent PCR applications, particularly in the field of prenatal diagnosis. Exclusion of contamination during extraction has become a major challenge since the introduction of real-time PCR. The results described here indicate that nearly complete automation of the DNA extraction, amplification, and detection steps can be achieved. This automated procedure could have implications for systematic analysis, such as RhD fe-

Automated Assay for Fetal DNA Analysis in Maternal Serum

To the Editor:

Since the first demonstration of the presence of cell-free fetal DNA in maternal plasma of pregnant women (1), many reports have been published, mainly about fetal sex and RhD determinations (2, 3). Although good results have been obtained with conventional PCR, real-time PCR is now the most widely used amplification method for fetal DNA analysis because it allows high sensitivity with a high degree of protection against contamination (4). Conventional PCR may produce false-positive results from contamination (5), whereas real-time PCR, because it is a closed-tube system, reduces the risk of false-positive results from carryover of PCR products (6, 7). During sample preparation, however, contamination can result either from cross-contamination between samples or from the operator, particularly if a very small amount of human target sequence has to be detected, such as cell-free fetal DNA in maternal serum.

We evaluated a fully automated sample preparation system (8) combined with real-time PCR. We extracted DNA from maternal serum by use of the MagNA Pure LC apparatus (Roche Biochemicals) with the Total Nucleic Acid LV reagent set (Roche) according to the manufacturer’s instructions. The eluted DNA and PCR reagents were automatically dispensed into PCR capillaries by an integrated PCR reaction set-up procedure. The operator only placed the reagents and samples in the apparatus.

In the first part of the study, cross-contamination between samples was evaluated. We introduced 1 mL of serum from a male and a female in an alternating pattern (Fig. 1A). DNA was eluted in 50 μL of elution buffer. We used 10 μL of each of the 32 eluates for real-time PCR amplification of the SRY gene (3). Two clearly distinguishable groups of curves were observed (Fig. 1B): as expected, the 16 extracted DNA samples from male serum gave positive results for the SRY gene, whereas all DNA extracts from the female serum gave negative results. Quantitative results for the male serum, expressed as crossing points (cp), defined as the maximum of the second derivative of the fluorescence curves, revealed a CV of 0.4% at a 30.6 cp value (n = 16).

In the second part of the study, 108 sera from pregnant women (mean gestational age, 11.7 weeks) were analyzed by the above fully automated procedure. Results were compared with those obtained using the conventional manual procedure (3). The results of the two methods were completely concordant. All sera from pregnant women carrying a male fetus were negative for the SRY gene (n = 62), whereas all sera from pregnant women carrying a male fetus were positive (n = 46). With the fully automated procedure, the concentration of male fetal DNA in maternal serum during the first trimester of pregnancy was estimated to range from 5.2 to 64.9 copies/mL (mean, 26.3 copies/mL). This result was similar to those obtained by others (9). The CV of the test in this range of concentration was ±2% (one sample analyzed 12 times during one analytical run; mean cp, 38.6; SD, 0.8 cp), which reflects the reproducibility of both DNA extraction and PCR assay.

Analysis of fetal DNA in maternal serum offers new possibilities for noninvasive prenatal diagnosis. A lack of contamination is crucial for subsequent PCR applications, particularly in the field of prenatal diagnosis. Exclusion of contamination during extraction has become a major challenge since the introduction of real-time PCR. The results described here indicate that nearly complete automation of the DNA extraction, amplification, and detection steps can be achieved. This automated procedure could have implications for systematic analysis, such as RhD fe-

Fig. 1. Evaluation of cross-contamination during DNA extraction using MagNA Pure LC instrument.

Thirty-two samples were processed during the same run. (A), 1 mL of either a male or a female serum was introduced in the sample cartridge in an alternating pattern. (B), all extracted DNA samples were analyzed for the presence of the SRY gene by real-time PCR.
tual genotype determination for all RhD-negative pregnant women, because it allows analysis of 30 samples in <3 h. The MagNA Pure LC instrument in combination with real-time PCR may be useful for quantitative analysis and for large-scale studies to determine whether fetal DNA quantification can be used as a marker for fetal trisomy 21 (10).

The MagNA Pure LC instrument was kindly provided by Roche Biochemicals (Meylan, France). We are indebted to Dr. Lavergne for reviewing the manuscript.

References

Jean-Marc Costa*
Pauline Ernault
M. Dassault Molecular Biology Laboratory Centre de Diagnostic Prénatal American Hospital of Paris

63 bd Victor Hugo
92200 Neuilly-sur-Seine, France

*Author for correspondence. E-mail jean-marc.costa@ahparis.org.

Method-dependent Changes in “HDL-Cholesterol” with Recombinant Apolipoprotein A\(^{\text{I-Milano}}\) Infusion in Healthy Volunteers

To the Editor:

Warnick et al. (1) recently reviewed the status of the measurement of HDL-cholesterol (HDL-C) and cautioned against the use of the new homogeneous methods, when atypical specimens may be present, without additional validation of the accuracy of the methods. We recently had the opportunity to analyze specimens from a clinical trial, which will serve as an excellent example of the warning given by these authors.

In a phase I clinical trial, 32 healthy volunteers were infused with a synthetic HDL comprising recombinant apolipoprotein (Apo) A\(^{\text{I-Milano}}\) and phospholipid [1-palmitoyl-2-oleoyl-sn-3-glycerophosphocholine (POPC)]. This cholesterol-free, HDL-like complex is referred to as ETC-216. The intent of the trial was to determine safety/tolerability before initiating a phase II study in patients with acute coronary syndromes. It has been suggested that ApoA-I\(^{\text{Milano}}\)/phospholipid infusions may rapidly stabilize atherosclerotic lesions by mobilizing vessel wall cholesterol.

Table 1. Measurement of HDL-C concentration by various homogeneous and precipitation methods.

<table>
<thead>
<tr>
<th>HDL-C method</th>
<th>Mean change from pretreatment, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran sulfate</td>
<td>24.7</td>
</tr>
<tr>
<td>Heparin manganese</td>
<td>20.9</td>
</tr>
<tr>
<td>Sigma EZ</td>
<td>7.2</td>
</tr>
<tr>
<td>Roche HDL Plus</td>
<td>−82.0</td>
</tr>
<tr>
<td>FPLC HDL Cholesterol</td>
<td>42.2</td>
</tr>
</tbody>
</table>

Table 1. Measurement of HDL-C concentration by various homogeneous and precipitation methods.

<table>
<thead>
<tr>
<th>HDL-C method</th>
<th>Mean change from pretreatment, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran sulfate</td>
<td>24.7</td>
</tr>
<tr>
<td>Heparin manganese</td>
<td>20.9</td>
</tr>
<tr>
<td>Sigma EZ</td>
<td>7.2</td>
</tr>
<tr>
<td>Roche HDL Plus</td>
<td>−82.0</td>
</tr>
<tr>
<td>FPLC HDL Cholesterol</td>
<td>42.2</td>
</tr>
</tbody>
</table>

Initial measurements of HDL-C concentrations before and 30 min after the start of the infusion gave divergent results when we compared results obtained with a polyethylene glycol/sulfated α-cyclodextrin-based method (Roche HDL Plus) to those obtained with separation of HDL by size-exclusion chromatography by fast protein liquid chromatography (FPLC) with online determination of cholesterol concentration (3). To further investigate this discrepancy, sera from a subset of seven patients (fasted males; doses of 50–100 mg ApoA-I\(^{\text{Milano}}\) protein/kg; infusion time, 1–1.3 h) were referred to the Core Laboratory for Clinical Studies for measurement of HDL-C by several homogeneous and traditional precipitation methods (see Table 1).

It is important to note that before treatment with ETC-216, each method reported similar HDL-C concentrations, suggesting agreement under normal conditions. Serum samples were obtained at 0.5 and 2 h after the start of the infusion and pooled for each patient because HDL-C values by FPLC were not different at these time points.

After treatment with ETC-216, the two precipitation methods based on dextran sulfate (M, 50 000) and heparin manganese gave similar results, with a decrease in HDL-C of 21–25%. The HDL-C concentrations obtained by the homogeneous methods were different from the precipitation methods and also quite different from each other. The antibody-based Sigma EZ method showed an increase in HDL-C similar to the in-