SD) was 13.9 ng/L. In this calculation, a value <5 ng/L was considered as “none detected” or a zero value. The manufacturer defined the detection limit as 5 ng/L, which is lower than our observation. The manufacturer also defined functional sensitivity of the assay (lowest concentration with a CV <20%) as 50 ng/L. However, in our experience, NT-proBNP values <45 ng/L are not reliable because when Diluent Universal (sample diluent for all Elecsys assay; Roche Diagnostics) was analyzed 10 times in a single batch with this assay, we observed a range of readings between 33 and 44 ng/L [mean (SD), 37 (4.1) ng/L; mean + 2 SD, 45.2 ng/L]. The within-run CV was 11%. In contrast, we observed excellent precision for the NT-proBNP assay in a specimen with a low NT-proBNP concentration of 60 ng/L (total CV, 4.9%; n = 10). Precision was further improved in another specimen with a NT-proBNP concentration of 100 ng/L (total CV, 2.1%; n = 10).

We observed no adsorption of NT-proBNP by the gel of the SST tubes in the 10 volunteers we studied (NT-proBNP range, 64–302 ng/L; five males and five females). The NT-proBNP values obtained in the specimens collected in the red-top tubes did not differ from those for the specimens collected in SST tubes at 0–1, 2, 5, 24, or 48 h. For example, the mean (SD) NT-proBNP concentration in the first volunteer was 64 (1.1) ng/L in specimens collected in the plain red-top tubes. The corresponding NT-proBNP value in the specimens collected in SST tubes was 61 (3.0) ng/L. After 48 h, the mean (SD) NT-proBNP concentration in the specimens collected in plain red-top tubes was 63 (1.0) ng/L, and the NT-proBNP concentration in the specimens collected in SST tubes was 64 (1.2) ng/L. These values are not significantly different by the two-tailed Student t-test at 95% confidence. A comparison of NT-proBNP concentrations in specimens collected in plain red-top tubes and in SST tubes from two volunteers with NT-proBNP concentrations of 125 ng/L (decision point) and 302 ng/L is shown in Fig. 1.

We also observed no adsorption of NT-proBNP by the gel in the 10 patients we studied. We studied nine patients with high NT-proBNP concentrations (range, 1424–12 851 ng/L) and in one patient near the cutoff value of 125 ng/L. In all cases, we observed no statistically significant difference in NT-proBNP values within 1 h of blood collection and after 48 h of storage of the specimens (both plain red-top tubes and SST tubes). For example, in one patient, the mean (SD) original NT-proBNP concentration of 5820 (47) ng/L was not statistically different from the mean (SD) NT-proBNP value of 5908 (100) ng/L observed after 48 h in a specimen stored in a SST tube. Another patient with an original mean (SD) NT-proBNP concentration of 120 (3.5) ng/L had a mean (SD) NT-proBNP value of 121 (4.2) ng/L after storage of specimen in a SST tube for 48 h at 4 °C. However, one limitation of this study is that blood was collected in SST tubes and parallel specimens were not collected in plain glass tubes in these patients.

Our previous studies on the stability of therapeutic drugs in specimens stored in SST tubes demonstrated that reductions in the values were more significant at low drug concentrations. Because adsorption of an analyte by the gel is time dependent, any adsorption of NT-proBNP by the gel should be reflected in significant decreases in NT-proBNP concentrations as a function of storage time. The adsorption of therapeutic drugs by the gel is time dependent, and a significant decrease in drug concentration can be observed within 2–4 h of storage (7, 9). We observed no statistically significant decrease in NT-proBNP concentrations over a 48 h period in specimens collected in SST tubes.

In conclusion, we believe that SST tubes manufactured by Becton Dickinson can be used for blood collection for routine analysis of NT-proBNP in both fresh and stored samples.

We thank Roche Diagnostics (Indianapolis, IN) for providing the NT-proBNP assays.

References

Physical Characteristics of Six New Thermocyclers, Dagmar Schoder,1 Alois Schmalwieser,2 Günther Schauberger,2 Matthias Kuhn,3 Jeffrey Hoofar,4 and Martin Wagner1 (1 Institute for Milk Hygiene, Milk Technology and Food Science, Veterinärplatz 1, 1210 Vienna, Austria; 2 Institute for Medical Physics and Biostatistics, Veterinärplatz 1, 1210 Vienna, Austria; 3 Congen Biotechnologie, Robert-Roessle Strasse 10, 13125 Berlin, Germany; 4 Danish Veterinary Institute, Bülowsvej 27, DK-1790 Copenhagen, Denmark; * author for correspondence: fax 43-1-25077-3590, e-mail dagmar.schoder@vu-wien.ac.at)

Since the publication of the first article describing PCR, thermocyclers have become a staple in academic and industrial laboratories (1). The thermocycler is a program-
Fig. 1. Temperature profiles of the six thermal cyclers.

Evaluation of the temperature profiles allowed us to group the six test cyclers into three categories. The temperature profiles of the category 1 thermocyclers (A and B) are depicted in panel A, those of the category 2 thermocyclers (D and E) are shown in panel B, and those of the category 3 thermocyclers (C and F) are shown in panel C. The in-tube temperature was measured in position D4. The temperature program (PCR RAPD short protocol) was as follows: 97 °C for 30s, 39 °C for 30s, and 72 °C for 30 s.
mable cycling incubator that performs repeated PCR steps of DNA denaturation, primer annealing, and primer elongation at defined intervals. Rapid heat transfer from the heating block to the in-tube sample liquid ensures a high efficiency of amplicon multiplication; therefore, a thermal processor should guarantee temperature uniformity for all samples within an individual run as well as run-to-run repeatability.

PCR-based protocols can give unsatisfying results (2, 3). Several collaborative studies have shown weak reproducibility with random amplified polymorphic DNA (RAPD) protocols (4, 5). One reason might be the influence of the thermocycler on amplification efficiency.

Despite its striking importance for PCR, the literature on thermocyclers is scarce. Some studies were published on the first generation of cyclers (6–8). Others determined the amplification efficiency but did not evaluate the physical characteristics (9, 10). The impact on PCR of the variation within thermocyclers, with regard to their thermocycling settings, has not been fully determined. The goal of this study was to define the physical characteristics of performance of the latest generation of thermocyclers and to discuss the influence of the physical properties on amplification efficiency.

Six new thermocyclers were selected for this performance study: (A) Gene Amp 9700 (Applied Biosystems), (B) Multicycler PTC 200 (MJ Research, Inc.), (C) Tgradient (Whatman Biometra GmbH), (D) Mastercycler gradient (Eppendorf Netheler-Hinz GmbH), (E) Touchgene (Techné, Inc.), and (F) Primus 96 (MWG AG Biotech). All experiments were performed with the lid temperature set to 105 °C and with the maximum ramp rate available.

The temperature was measured in 0.2-mL PCR tubes (MicroAmp; Applied Biosystems) containing 50 μL of distilled water. Fast-response type T microthermocouples (copper/constantan; i.d., 0.6 mm; isolated with polytetrafluoroethy, RS Components) and a 263A Data Bucket data logger (Fluke Cooperation) were used. The data logger was connected to a personal computer via the RS232 with use of IEEE-488 as communication language. A special software package (ScanScape; Fluke Cooper.) enabled data collection and data storage. The setup of the experiments allowed the measurement of the in-tube temperature at 13 block positions (A1, H1, B2, G2, D4, A6, F6, C7, E10, B11, G11, A12, and H12).

The measurement unit was calibrated in accordance with specifications established for legal metrology, which documents traceability to national standards. The calibration was carried out by an officially accredited calibration authority (TESTO Comp.) at the following three temperature set points: 39, 72, and 97 °C. The certified temperature accuracy and reproducibility were ± 0.3 °C and ± 0.1 °C, respectively.

Each thermocycler was programmed to perform a dynamic temperature protocol (PCR RAPD short) that corresponded to a typical RAPD assay: 30 cycles of 97 °C for 30 s, 39 °C for 30 s, and 72 °C for 30 s (11). Evaluation of the temperature profiles allowed us to group the six test cyclers into three categories. Cyclers A and B were assigned to category 1 (Fig. 1A), cyclers D and E were assigned to category 2 (Fig. 1B), and cyclers C and F were assigned to category 3 (Fig. 1C).

The performance of category 1 cyclers resembled an idealized chart (Fig. 1A). The in-tube temperatures precisely followed the programmed temperatures. The mean PCR step duration (\( \text{Step}_{\text{mean}} \)) was calculated to be 30 s for cycler B and 31 s for cycler A (Table 1).

Category 2 cyclers (D and E) had short over-/under-shooting phases before performing each PCR step (Fig. 1B). The \( \text{Step}_{\text{mean}} \) was 38 s for cycler D, which means that the time for one cycle of denaturation, annealing, and elongation was extended by up to 30%. In cycler E, on the other hand, the annealing and elongation phases were reduced by up to 10%, whereas the denaturation was extended by 7% (Table 1).

The temperature profile of cyclers C and F (category 3) did not correspond to the programmed temperature protocol. Both failed to achieve a temperature plateau for the denaturation, annealing, and elongation phases (Fig. 1C). As a consequence, the \( \text{Step}_{\text{mean}} \) was shorter than with the other thermocyclers: 12 s for cycler C and 15 s for cycler F.

The performance of a thermocycler has a critical influence on PCR efficiency. Interlaboratory trials comparing up to 33 thermocyclers of various makes and models reported a failure to obtain reproducible banding patterns by RAPD assays (5, 9). Use of inappropriate cyclers was mentioned as one of the main reasons for these results; this was perhaps attributable to differences in age, temperature control options, and calibration status (9). The physical characteristics of performance, however, were never examined in detail.

To circumvent these shortcomings, we exclusively com-

---

![Table 1. Dynamic temperature protocols (duration of the denaturation, annealing, and elongation steps) of the six cyclers (A–F) performing the PCR RAPD short protocol.](https://example.com/table1.png)

<table>
<thead>
<tr>
<th>Step</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denaturation</td>
<td><strong>Length of step, s</strong></td>
<td>32</td>
<td>29</td>
<td>13</td>
<td>37</td>
<td>32</td>
</tr>
<tr>
<td>Relative difference, %</td>
<td>+7</td>
<td>−3</td>
<td>−57</td>
<td>+23</td>
<td>+7</td>
<td>−50</td>
</tr>
<tr>
<td>Annealing</td>
<td><strong>Length of step, s</strong></td>
<td>31</td>
<td>32</td>
<td>12</td>
<td>39</td>
<td>27</td>
</tr>
<tr>
<td>Relative difference, %</td>
<td>+3</td>
<td>+7</td>
<td>−60</td>
<td>+30</td>
<td>−10</td>
<td>−33</td>
</tr>
<tr>
<td>Elongation</td>
<td><strong>Length of step, s</strong></td>
<td>31</td>
<td>29</td>
<td>11</td>
<td>37</td>
<td>28</td>
</tr>
<tr>
<td>Relative difference, %</td>
<td>+3</td>
<td>−3</td>
<td>−63</td>
<td>+23</td>
<td>−7</td>
<td>−66</td>
</tr>
</tbody>
</table>

a The length of each step (denaturation, annealing, and elongation) is the length of time that the sample temperature was held constant (temperature changes were less than ±0.3 °C) for that step. Positive relative difference values indicate an increase and negative values a decrease in step length compared with the cycler settings for the RAPD short protocol (denaturation at 97 °C for 30 s, annealing at 39 °C for 30 s, and elongation at 72 °C for 30 s).

b \( \text{Step}_{\text{mean}} \) was calculated as the mean duration of all PCR steps (denaturation, annealing, and elongation) and refers to the actual time that the sample temperature was at the selected temperature.
pared new thermocyclers of the latest generation. By embedding a fast-response microthermocouple inside a PCR reaction tube, we were able to study the thermodynamic process from the block via the tube to the PCR sample in more detail. This physical evaluation allowed us to distinguish accurate performers (category 1 and 2 cyclers) from less accurate performers (category 3 cyclers).

At the denaturation step, during the first 15 s the thermal inhomogeneities became most evident. Mai et al. (12) showed that the minimum denaturation temperatures that enabled amplification were 86–88 °C. The best results were obtained with denaturation temperatures between 91 and 94 °C (12). We found that category 3 cyclers (C and F) failed to reach the critical temperature of 90 °C for 8 and 10 s, respectively, after the start of timing of this step (data not shown). Consequently, these less-accurate performers may not reach adequate denaturation temperatures during short denaturation holds. We concluded that false-negative PCR results would most likely be caused by insufficient melting of the template DNA. Because of the less stringent annealing temperatures and shorter primers used, RAPD assays might be even more sensitive to temperature inhomogeneities than conventional PCR.

We thank Kurt Wimmer for assistance with designing and constructing the temperature recording unit; we also thank Drs. Martin D’Agostino and Nigel Cook (Central Science Laboratory, York, United Kingdom) for careful reading of this manuscript. This project was supported by EC Grant QLK1-CT-1999-00226.

Reproducibility of Blood Markers of Oxidative Status and Endothelial Function in Healthy Individuals, Pascale G.A. Van Hoydonck,* Evert G. Schouten, and Elisabeth H.M. Temme (University of Leuven, Department of Public Health, Division of Nutritional Epidemiology, Kapucijnenvoer 33, B-3000, Leuven, Belgium; * author for correspondence: fax 32-16-336884, e-mail Pascale.Vanhoydonck@med.kuleuven.ac.be)

Oxidative processes and endothelial cell dysfunction play an important role in the etiology of atherosclerosis. Oxidative modification of LDL in the subendothelial space of the vessel wall is thought to be important in the initiation of atherosclerosis. Oxidized LDL (OxLDL) may not only contribute to foam cell generation, but also stimulate the synthesis of adhesion molecules by endothelial cells. Expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) promotes the adherence and migration of new monocytes through the endothelial barrier to the subendothelial space (1, 2). Finally, some OxLDL will diffuse back from the atherosclerotic arterial wall to the blood, in which it can be measured.

Interest is growing in the measurement of markers of atherosclerosis to predict disease risk and to investigate effects of different interventions. Data on the within-subject variation of these markers are necessary to adequately estimate the required sample sizes for intervention trials and/or the number of blood samples needed to obtain a stable estimate of typical concentrations. However, data on the reproducibility (i.e., within-subject and analytical variation) of oxidative and endothelial markers are still sparse. We therefore investigated the reproducibility of several diagnostic markers of oxidative processes (OxLDL, the endogenous antioxidants bilirubin and uric acid, and ferritin) and endothelial function (sICAM-1, sVCAM-1, and vWF) in healthy individuals. The markers were measured in samples taken on three different occasions within 1 week, and in men and women of different ages.

The study population consisted of 25 volunteers (12 men and 13 women; age range, 26–58 years; mean, 41 years). All were healthy as assessed by a medical questionnaire and were not taking any medications known to affect hemostatic values. The Ethics Committee approved the study protocol, and all participants gave informed consent before participation.

Three blood samples were collected within 1 week (day 0, day 3 or 4, and day 7) between 0800 and 1000 after an overnight fast (from 2200), and participants were asked to abstain from drinking alcohol during the day before blood sampling. All venipunctures were performed for each participant at the same hour of the day. Participants were