when stored at room temperature, or refrigerated for up to 14 days, or frozen for up to six months.
* Thaw frozen samples at 60 °C for 10 min.
* Centrifuge samples at 1000 × g for 2 min.
* Analyze within two weeks from samples from patients taking vitamin C because long-term storage allows conversion of exogenous ascorbate to oxalate in all storage methods studied.
* Avoid metal containers or caps and nitric acid as a preservative.

Plasma

This method is also sensitive enough to measure oxalate in the normal plasma range. Our plasma results of 2.5 μmol/L in healthy subjects agree with GC and isotope dilution techniques (13, 14). We changed the conditions for filtering plasma samples because our data showed poor recoveries of oxalate through the Centriflo cones at the pHs used by Kasidas and Rose (12). The 96% recovery we obtained is quite acceptable, but corrections could be made by using isotopes. Recovery of oxalate added to filtrates was also acceptable, and stability was good in filtrates stored at pH 3.5–4.0 at −20 °C for the two to three months tested.

References

CLIN. CHEM. 37/7, 1235–1244 (1991)

One-Step Chromogenic Equivalent of Activated Partial Thromboplastin Time Evaluated for Clinical Application

Gabrielle A. E. Ponjee, Huib L. Vader, Piet J. de Wild, Ger W. T. Janssen, and Fedde van der Graaf

We evaluated the clinical usefulness of a recently developed semi-automated one-step chromogenic equivalent of activated partial thromboplastin time (APTT; Behring). This simple test is easily adaptable for automation. Generally, the results with this chromogenic one-step APTT were at least as precise as those obtained with comparative coagulometric methods. The chromogenic one-step APTT showed, both in vitro and in vivo, adequate sensitivity to congenital intrinsic factor deficiency but no sensitivity to Factor VII deficiency. Unlike a two-step coagulometric APTT (Dade), the one-step chromogenic APTT seemed sensitive to activation products of the contact system, which are present in immunoabsorbed factor-deficient plasma. The in vitro sensitivity of the chromogenic APTT to heparin was comparable with that of a coagulometric APTT, but the sensitivity to heparin in patients' samples differed slightly. The chromogenic APTT is relatively insensitive to anomalies in the fibrinogen—fibrin conversion. Finally, we observed discrepancies between the chromogenic and coagulometric APTT results for plasma of patients with disseminated intravascular coagulation. We conclude that this one-step chromogenic APTT warrants further evaluation for possible use as a routine test for the clinical laboratory.

Additional Keyphrases: coagulation · fibrinogen—fibrin

A one-step partial thromboplastin time was first introduced by Langdell et al. (1) for the diagnosis of hemophilia. The term "partial" was used because, in contrast to complete thromboplastins, partial thromboplastins could not correct for the prolonged clotting time in plasma of hemophiliacs. The partial thromboplastin times were long and showed considerable variation.
because of inadequate activation at the relatively small surface area of the glass tubes in which the tests were performed. Waaler (2), who investigated the contact activation process of plasma, introduced kaolin and Celite, respectively, to initiate and accelerate activation. Furthermore, the extraction procedure for the preparation of cephalin became standardized (3).

Margolis (4), in his study on the initiation of blood coagulation by glass, kaolin, and various forms of silica, separated the process of contact activation from those phases of clotting that required calcium for completion. This two-step partial thromboplastin time, which included a pre-incubation period for optimum contact activation, appeared to give the most reproducible results and the shortest clotting times (5, 6). The activated partial thromboplastin time (APTT) gained popularity because several independent studies indicated that the APTT was a reliable, reproducible test to monitor heparin therapy (7–10).1 Meanwhile, the APTT test has undergone numerous modifications. Different types of cephalin have been suggested, recommended pre-incubation times have ranged from 2 to 10 min, and the methods to detect the fibrin clot have varied from the wire-loop and tilt-tube techniques to automated clot timers.

Nowadays, the APTT assay is widely used to monitor patients receiving heparin therapy and to screen for deficiency of intrinsic coagulation factor. Like all coagulation tests, the APTT suffers from a lack of standardization, although several efforts toward this goal have been undertaken. Major drawbacks are that (a) the results depend strongly on the method used to detect the fibrin clot (manual, conductometric, or turbidimetric) and (b) the results are sensitive to interferences by the fibrin polymerization process. With the introduction of specific chromogenic peptide substrates for serine proteases, as with the factors of the clotting system, an alternative to classical clot detection techniques has become available (11–14). Assays based on the use of thrombin-specific chromogenic peptide substrates have been proposed as the chromogenic equivalents of prothrombin time (PT) and of APTT. Coagulation assays based on this principle are easier to standardize than the conventional clotting assays. In addition, their simplicity and adaptability to automated instruments make the use of chromogenic substrates for coagulation assays very attractive.

Although several papers have described the usefulness of the chromogenic PT, little has been reported on the chromogenic APTT. Recently Behring (Behringwerke AG, Marburg, F.R.G.) introduced a new chromogenic APTT as an alternative to the traditional clotting APTT. In this assay, sulfatides are used to initiate the intrinsic clotting system. Because sulfatides are very potent activators of the contact system, a (separate) pre-incubation period was not necessary, making this one-step chromogenic APTT suitable for automation. Accordingly, however, the coagulation times of this test are much longer than those of the conventional two-step procedures.

Here, we investigate whether this one-step chromogenic equivalent of the APTT can be used as an alternative to the conventional two-step APTT. We evaluate its usefulness in determining deficiencies of intrinsic coagulation factors and in monitoring heparin therapy, and compare the results with those obtained by a two-step coagulometric APTT.

Materials and Methods

Samples

Citrated plasma was prepared by mixing nine volumes of freshly drawn blood obtained by venipuncture with one volume of sodium citrate (106 mmol/L) in polypropylene tubes. After centrifuging the blood (10 min, 1500 × g), we assayed the plasma without delay or froze it at −70 °C in polypropylene vials. "Normal plasma" was pooled citrated plasma collected from 44 apparently healthy donors (27 men and 21 women, ages 22 to 48 years, average 30 years) who had not taken any medication (including aspirin and oral contraceptives). The plasma was pooled and frozen at −70 °C in 0.5-mL aliquots in polypropylene vials. The "normal plasma" used to determine the repeatability and reproducibility of the APTT was obtained from one healthy donor.

Abnormal plasma for determining the repeatability and reproducibility of the APTT was obtained from a patient treated with oral anticoagulants. Abnormal plasma used for determining the repeatability of intrinsic clotting factors was normal plasma diluted with an equal volume of isotonic saline (NaCl 154 mmol/L).

Coagulation-factor-deficient plasma, prepared by immunoabsorption, was obtained from Behring. Plasmas congenitally deficient in factor VIII (FVIII) and prekallikrein (PK) were obtained from Sigma Chemical Co., St. Louis, MO. Plasmas congenitally deficient in FVIII (used in sensitivity experiments), FIX, FXI, FXII, and high-molecular-mass kininogen (HMWK) were obtained from George King Biomedical (Overland Park, KS). Congenital FVII-deficient plasma was obtained from Organon (Durham, NC).

Reference Values

The reference sample group used for determining the reference limits of the one-step chromogenic APTT comprised the 44 apparently healthy blood donors and 76 outpatients who did not use any medication and who had no known hematological or coagulation disorders. We determined the reference limits in accordance with the IFCC recommendation on the statistical treatment of collected reference values (16), using the REFVAL program (16). The lower and upper reference limits and their 90% confidence intervals were 83 (80–88 s) and 125 s (121–131 s), respectively. Consequently, we used 83–125 s as the reference interval for the one-step

1 Nonstandard abbreviations: APTT, activated partial thromboplastin time; PT, prothrombin time; F, clotting factor; PK, prekallikrein; HMWK, high-molecular-mass kininogen; and PDP, fibrinogen degradation products.
Chromogenic APTT. The reference range for the coagu-
lometric APTT was 27–34 s.

Patients
Patients taking heparin therapy received heparin by
continuous intravenous infusion. Most of them were
repeatedly treated with oral anticoagulants within 12 h after
initiation of the heparin therapy; a blood specimen
was obtained from each before they took the oral anti-
cogulants. In this set of samples we measured the
chromogenic and coagulometric APTT as well as the
heparin concentration.

Assays
APTT assay. The reagent used for the photometric
APTT method was Partochrom (Behringwerke). This
reagent contains human phospholipids and sulfates as
contact activators. The chromogenic substrate for
thrombin in this reagent is BCP-100 (Tos-Gly-Pro-Arg-
5-amino-2-nitrobenzoic acid-isopropylamide). The
chromogenic APTT assay was performed with the Behring
Chromotimer. According to the manufacturer’s recom-
pendations, we added 250 μL of reagent to 25 μL of
patient’s plasma.

The Chromotimer is a semi-automated coagulation
analyzer, consisting of an incubation block (37 °C) and a
microprocessor-controlled four-channel photometer
connected to a microcomputer. The APTT test is based on
determining the time necessary to increase absorbance
by 0.1 A after addition of the reagent. The concentration
of fibrinogen in plasma is usually between 5 and 15
μmol/L; in the assay with Partochrom, the plasma is
diluted 11-fold, yielding a concentration of 0.5 to 1.5
μmol/L. The concentration of the chromogenic substrate
in the assay is 45 μmol/L. Because the Michaelis con-
stants of thrombin for the chromogenic substrate and for
fibrinogen are comparable, the excess of chromogenic
substrate will competitively inhibit the thrombin-medi-
dated conversion of fibrinogen to fibrin. Given the con-
tions for determining the end point of the reaction, when
about 20% of the chromogenic substrate is cleaved,
fibrinogen present in low and normal concentrations is
unlikely to have any effect on the results of this APTT.

The coagulometric APTT was measured with Actin-
Activated Cephaloplastin Reagent (Dade, Dudingen,
Switzerland), which contains rabbit brain cephalin and
eellagic acid, by a manual til-tube technique. We added
100 μL of the reagent to 100 μL of patient’s plasma,
pre-incubated the mixture at 37 °C for 180 s, then added
100 μL of 20 mmol/L CaCl₂ solution and measured the
clotting time.

Heparin assay. Heparin concentration was deter-
minal with the Berichrom heparin assay (Behring-
werke). We incubated 25 μL of plasma with 25 μL of
FXa solution containing dextran sulfate for the neutral-
ization of Platelet Factor 4, and 25 μL of antithrombin
III (in excess) for 1 min at 37 °C. Residual FXa activity
was measured photometrically after a chromogenic sub-
strate had been added.

Intrinsic clotting factor assay. During preliminary
experiments, we found better repeatability if we pre-
diluted the patient’s plasma fivefold with isotonic saline
and added 50 μL of this diluted plasma to the reaction
mixture (50 μL of factor-deficient plasma and 500 μL of
Partochrom reagent), instead of the 10 μL recommended
by the manufacturer. We used this modification
throughout our experiments. The concentration of in-
trinsic clotting factor was read from a five-point calibra-
tion curve, constructed from serial dilutions of normal
plasma with isotonic saline to final concentrations of
100%, 50%, 25%, 12.5%, and 6.25% of the original
values, respectively.

The coagulometric intrinsic clotting assay was carried
out semi-automatically with a Schnitter and Groen coag-
ulometer (Amelung KG, Lieme, F.R.G.), incubating 100
μL of fivefold-prediluted patient’s plasma with 100 μL of
Actin at 37 °C. After 4 min, 100 μL of 20 mmol/L CaCl₂
reagent was added to the mixture and the clotting time
was measured. The concentration of intrinsic clotting
factor was calculated from the calibration curve.

Analytical Performance

Stability of reagent. The stability of the Partochrom
reagent was tested by dissolving it according to the
manufacturer’s instructions and incubating it at 37 °C.
After 0, 3, 6, and 8 h, we measured the APTT of normal
plasma and of normal plasma diluted two- and fourfold
with isotonic saline. We repeated this procedure after
incubating the Partochrom reagent for 24 h at 4 °C. The
chromogenic APTT of different concentrations of normal
plasma did not change significantly at the different
incubation times or after the incubation of Partochrom
for 24 h at 4 °C.

Effects of lipemia, hemoglobin, and bilirubin on the
performance of the APTT. To test the influence of
lipemia on the chromogenic APTT method, we added
increasing amounts of a milky fat emulsion (Intralipid
10%; Kabi Vitrum, Stockholm, Sweden) to normal
plasma, up to 10/90 (by vol). Interference by hemoglobin
or bilirubin was determined by adding either a constant
amount of a hemoglobin-containing solution or a bili-
rubin standard solution (unconjugated bilirubin in an
albumin-containing Tris- HCl buffer, pH 7.4; RIVM,
Bilthoven, The Netherlands) (17).

Sensitivity of the APTT to intrinsic clotting factor
deficiency. We assessed the sensitivity of the chromog-
omic and coagulometric APTT tests to intrinsic clotting
factor deficiency by measuring the APTT in normal
plasma (100% coagulation factor activity) mixed with
genital or immunoabsorbed factor-deficient plasma
(≤1% activity) in different ratios (final proportions of
75%, 60%, 50%, 40%, 30%, and 15%). The sensitivity of
the APTT to a PK-deficiency was determined only with
congenital PK-deficient plasma. The in vitro sensitivity
of the APTT to factor deficiency was defined as the
greatest concentration of an intrinsic clotting factor that
prolonged the APTT beyond the upper reference limit.

Sensitivity of the APTT to heparin. The sensitivity of
the chromogenic and coagulometric APTT to heparin in
vitro was tested by measuring the APTT in normal
plasma to which increasing amounts of heparin had been added. The heparin used in this experiment, obtained from Rousell-Uclaf (Paris, France), is the same as that used in our clinic for therapeutic treatment. The curve for log-linear APTT vs heparin activity was plotted and the correlation between the APTT values and plasma heparin concentration was calculated by linear least-squares regression analysis. The heparin-APTT doubling concentration (Hd), defined as the increment in plasma heparin activity required to double any APTT value, was calculated as follows: 

\[ \text{Hd} = \frac{\ln 2}{h} \]

where \( \ln 2 \) is the natural logarithm of 2, and \( h \) is the slope of the log-linear APTT-heparin curve (18).

The influence of heparin neutralization on the chromogenic APTT was determined by adding protamine-HCl (Hoffmann-La Roche BV, Mijdrecht, The Netherlands) or Heparsorb (triethylaminoethyl-cellulose; Organon, Oss, The Netherlands), an ion-exchange-type neutralizer, to in vitro heparinized normal plasma.

**Influence of increased coagulation factors and fibrinogen degradation products (FDP).** The influence of increased concentrations of FVII, FII, fibrinogen, and FDP on the chromogenic APTT was determined by adding increasing amounts of purified FVII, FII, fibrinogen (all from Sigma Chemical Co.), and FDP to normal plasma (10/90 by vol, final proportions). The influence of increased FXIII was tested in plasma samples from six pregnant women in their third trimester. Final concentrations of clotting factors in the test solution were measured as described above. FDP were prepared by digesting purified plasminogen-rich fibrinogen (Kabi Vitrum) with streptokinase (2000 kilo-int. units/L final concentration; Streptase, Behringwerke AG) (19). The reaction was stopped after 4 h by adding aprotinin (Trasyrol, 250 kilo-kallikrein inhibition units/L final concentration; Bayer, Leverkusen, F.R.G.). FDP were measured semiquantitatively in serum by latex agglutination (Thruomo-Wellcotest; Wellcome Diagnostics, London, U.K.).

**Results**

**In Vitro Studies**

**Precision.** Within-run and between-run CVs of the chromogenic APTT measured in normal plasma were 2.4% and 3.8%, respectively; measured in abnormal plasma, these were 2.0% and 4.5%, respectively. Table 1 summarizes the repeatability of the chromogenic and coagulometric assays of the intrinsic factors in normal plasma and abnormal plasma. Both congenital and immunoadsorbed factor-deficient plasma were used for these tests. The repeatability of the intrinsic clotting factor assay, determined with the chromogenic APTT in normal plasma and abnormal plasma, ranged from 2.3% to 7.6% (except for PK determined in normal plasma) and was comparable with that of the coagulometric intrinsic factor assay (Actin). For HMWK and PK, the repeatability of the chromogenic assay seemed even better. As Table 1 also shows, the source of the factor-deficient plasma—either immunoadsorbed or congenital—had no influence on the results.

**Effect of lipemia, hemoglobin, and bilirubin on APTT.** We tested the effects of increasing concentrations of triglycerides, hemoglobin, or bilirubin to normal plasma (Table 2). Triglyceride concentrations up to 4.7 mmol/L, hemoglobin concentrations up to 0.4 mmol/L,

<table>
<thead>
<tr>
<th>Table 1. Repeatability of Chromogenic and Coagulometric Factor Determination in Normal Plasma and Abnormal Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal plasma</strong></td>
</tr>
<tr>
<td><strong>Immunoadsorbed</strong></td>
</tr>
<tr>
<td><strong>deficient</strong></td>
</tr>
<tr>
<td><strong>plasma</strong></td>
</tr>
<tr>
<td><strong>Partochrom</strong></td>
</tr>
<tr>
<td><strong>Actin</strong></td>
</tr>
<tr>
<td><strong>FVIII</strong></td>
</tr>
<tr>
<td>Mean, %</td>
</tr>
<tr>
<td>CV, %</td>
</tr>
<tr>
<td><strong>FIX</strong></td>
</tr>
<tr>
<td>Mean, %</td>
</tr>
<tr>
<td>CV, %</td>
</tr>
<tr>
<td><strong>FXI</strong></td>
</tr>
<tr>
<td>Mean, %</td>
</tr>
<tr>
<td>CV, %</td>
</tr>
<tr>
<td><strong>FXII</strong></td>
</tr>
<tr>
<td>Mean, %</td>
</tr>
<tr>
<td>CV, %</td>
</tr>
<tr>
<td><strong>HMWK</strong></td>
</tr>
<tr>
<td>Mean, %</td>
</tr>
<tr>
<td>CV, %</td>
</tr>
<tr>
<td><strong>PK</strong></td>
</tr>
<tr>
<td>Mean, %</td>
</tr>
<tr>
<td>CV, %</td>
</tr>
</tbody>
</table>

* a,b = n = 16 except a = 6, b = 10
and bilirubin concentrations up to 300 μmol/L did not influence the chromogenic APTT. Higher concentrations of these substances prolonged the chromogenic APTT.

**Sensitivity of the APTT to factor deficiency.** The results of the in vitro studies on the sensitivity of the chromogenic APTT to deficiencies of FVIII, FIX, FXI, FXII, HMWK, and PK are shown in Figure 1 and Table 3. When immunoabsorbed factor-deficient plasma was mixed with normal plasma, the decreasing concentrations of FXI, FXII, and HMWK initially led to a paradoxical decrease of the chromogenic APTT (Figure 1, top). Hence, the sensitivity of the assay to these factors appeared to be low. We saw the same phenomenon with FIX when we performed this experiment with another batch of immunoabsorbed FIX-deficient plasma. Repeating the experiments with use of the congenital factor-deficient plasma improved the sensitivity considerably. Now a decreasing factor concentration did not shorten the chromogenic APTT (Figure 1, bottom). We repeated these experiments with the coagulometric two-step APTT and found that decreasing factor concentrations led to the expected prolongation of the APTT. Therefore, immunoabsorbed factor-deficient plasmas possibly contain products that shorten the one-step chromogenic APTT but not the two-step coagulometric APTT.

Table 3 summarizes the results of these in vitro sensitivity studies. The sensitivity of the one-step chromogenic APTT and that of the two-step coagulometric APTT were comparable with respect to deficiencies of FIX, FXII, and HMWK (and PK). Regarding deficiencies of FVIII and FXI, the in vitro sensitivity of the coagulometric APTT appeared to be better.

Recently Duncan et al. (20) reported that the chromogenic APTT, unlike the coagulometric APTT, is sensitive to FVII deficiency. However, by using Partochrom to measure chromogenic APTT in mixtures of normal

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**Table 2. Effect of Triglycerides, Hemoglobin, and Bilirubin on the Chromogenic APTT**

<table>
<thead>
<tr>
<th>Triglycerides, mmol/L</th>
<th>1.31</th>
<th>109</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.56</td>
<td>108</td>
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<tr>
<td></td>
<td>3.61</td>
<td>112</td>
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<tr>
<td></td>
<td>4.71</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>5.91</td>
<td>124</td>
</tr>
<tr>
<td>Hemoglobin, mmol/L</td>
<td>0</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>122</td>
</tr>
<tr>
<td>Bilirubin, μmol/L</td>
<td>13</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>113</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>213</td>
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<td>413</td>
<td>124</td>
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<tr>
<td></td>
<td>513</td>
<td>128</td>
</tr>
</tbody>
</table>

* Reference range 83–125 s.
Table 3. In Vitro Sensitivity for Intrinsic Factor Deficiencies: One-Step Chromogenic APTT vs Two-Step Coagulometric APTT

<table>
<thead>
<tr>
<th>Deficient factor</th>
<th>Chromogenic APTT</th>
<th>Coagulometric APTT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immuno-adsorbed</td>
<td>Congenital</td>
</tr>
<tr>
<td></td>
<td>deficient plasma</td>
<td>deficient plasma</td>
</tr>
<tr>
<td>FVIII</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>FIXI</td>
<td>45</td>
<td>30</td>
</tr>
<tr>
<td>FXI</td>
<td>*</td>
<td>35</td>
</tr>
<tr>
<td>FIXI</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>HMWK</td>
<td>20</td>
<td>55</td>
</tr>
<tr>
<td>PK</td>
<td>ND</td>
<td>55</td>
</tr>
</tbody>
</table>

* No prolongation of the APTT registered.

plasma with increasing quantities of congenital FVII-deficient plasma, we were not able to show an increase in the chromogenic APTT. Indeed, the chromogenic APTT of congenital FVII-deficient plasma was within the reference range. Therefore, we conclude that the Partochrom chromogenic APTT is not sensitive to an FVII deficiency.

**Sensitivity of the APTT to heparin.** The in vitro sensitivity of chromogenic APTT to heparin was tested by adding increasing amounts of heparin to normal pooled plasma. Both the chromogenic APTT and the coagulometric APTT exhibited a log-linear relationship with the heparin concentration (Figure 2). The slopes of the relationships were 1.77 (SD 0.28) mL/int. unit for the chromogenic APTT and 2.13 (SD 0.32) mL/int. unit for the coagulometric APTT. The heparin-APTT doubling concentrations, which refer to the increase in plasma heparin concentration required to double the APTT value, were 0.39 (SD 0.05) int. unit/mL for the chromogenic APTT and 0.33 (SD 0.05) int. unit/mL for the coagulometric APTT. Given the closeness of these values, we conclude that the chromogenic APTT and the coagulometric APTT show a similar sensitivity to heparin in vitro.

Addition of Heparsorb, 7 g/L, to in vitro heparinized plasma (maximum heparin concentration tested was 1.5 int. units/mL) completely normalized both the chromogenic and the coagulometric APTT values. Protamine HCl, 0.5 int. unit/mL, neutralized heparin concentrations as great as 0.8 int. unit/mL. A higher concentration of the protamine (according to the manufacturer, 1 int. unit/mL is necessary to neutralize 1 int. unit of heparin per milliliter) prolonged both the chromogenic and the coagulometric APTT (>30 s and >5 s, respectively), which makes protamine HCl less suitable than Heparsorb as a heparin neutralizer.

**Influence of increased amounts of coagulation factors and FDP.** The results of the experiments on the influence of increased amounts of FVIII, FVII, FII, fibrinogen, and FDP on the APTT are shown in Table 4. The presence of high concentrations of FVII and FII influenced neither the chromogenic nor the coagulometric APTT. However, very high concentrations of FII (>215%) induced a slight prolongation of the chromo-

Table 4. Effect of Increasing Amounts of FVII, FII, FVIII, Fibrinogen, and FDP on Chromogenic APTT and Coagulometric APTT

<table>
<thead>
<tr>
<th></th>
<th>Chromogenic</th>
<th>Coagulometric</th>
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<tr>
<td></td>
<td>Reference range</td>
<td>APTT, s</td>
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<tr>
<td></td>
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<td>27–34</td>
</tr>
<tr>
<td>FVII, %*</td>
<td>90</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>190</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>290</td>
<td>111</td>
</tr>
<tr>
<td>FII, %*</td>
<td>90</td>
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<tr>
<td></td>
<td>215</td>
<td>112</td>
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<tr>
<td></td>
<td>340</td>
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<td>FVIII, %*</td>
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</tr>
<tr>
<td></td>
<td>140</td>
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</tr>
<tr>
<td></td>
<td>310</td>
<td>98</td>
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<tr>
<td>Fibrinogen, g/L</td>
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<td>110</td>
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<tr>
<td></td>
<td>3.4</td>
<td>108</td>
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<td></td>
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<td></td>
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<td>117</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>117</td>
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<tr>
<td>FDP, mg/L</td>
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</tr>
<tr>
<td></td>
<td>50–100</td>
<td>109</td>
</tr>
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<td>114</td>
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<tr>
<td></td>
<td>800–1600</td>
<td>122</td>
</tr>
</tbody>
</table>

* Value in normal plasma is 100%.

Fig. 2. In vitro sensitivity of the chromogenic (C) and coagulometric (*) APTT to heparin

Different amounts of heparin were added to normal pooled plasma. The horizontal lines denote the limits of the reference interval.
The chromogenic APTT, measured in plasma of five pregnant women with FVIII concentrations ranging from 130% to 310% of normal, was comparable with the chromogenic APTT measured in normal plasma. A marked influence of high concentrations of FVIII on the performance of the chromogenic APTT therefore seems unlikely. As shown in Table 4, increased concentrations of fibrinogen or FDP had only a slight effect on the chromogenic APTT. Very high concentrations of FDP (>800 mg/L) produced a more pronounced effect (prolongation >10 s).

Patients' Studies

We compared the chromogenic APTT assay with the coagulometric APTT assay in plasma samples from 44 apparently healthy individuals and 50 patients treated with oral anticoagulants (Figure 3). For the comparison of results for the healthy individuals, r = 0.48 (Spearman's rank correlation). For 42 of the samples from the healthy individuals, results were within the reference ranges of both methods; one sample had a normal coagulometric APTT and an abnormal chromogenic APTT, and the remaining sample had prolonged APTTs with both methods. Coagulation factor assays revealed FXII deficiency in both samples (53% and 22% of normal, respectively). For the 50 patients taking oral anticoagulants, the correlation between assays (Spearman's rank correlation) was stronger: r = 0.71.

Comparison of the chromogenic (y) and coagulometric (x) APTT ratios in samples from 30 other patients on heparin therapy produced an orthogonal regression relationship of \( y = 0.58x + 0.64 \) (Spearman's r = 0.29, r = 0.79). The ratios were calculated by dividing the APTT of a patient's plasma by the APTT of normal plasma (104 s for the chromogenic APTT and 30 s for the coagulometric APTT). In general, the chromogenic APTT appeared to be slightly less sensitive to heparin than the coagulometric APTT in terms of APTT ratios. In the 16 patients for whom enough sample was available for all these comparisons, neither the chromogenic APTT ratio nor the coagulometric APTT ratio correlated well with the heparin concentration (Spearman's r was 0.47 and 0.53, respectively).

To compare the diagnostic sensitivity to intrinsic factor deficiency of the chromogenic APTT method and a coagulometric APTT assay, we performed both tests in plasma of 24 patients with mild congenital intrinsic factor deficiency; their FVIII values varied from 2% to 55% of normal, FXII 19–53%, and FXI 3–42% (Figure 4). Spearman's rank coefficient of correlation was 0.65. In all but one sample the factor deficiency was detected by both APTT methods. The chromogenic APTT seemed more sensitive to an FXII deficiency than was the coagulometric APTT, shown by a prolongation of the chromogenic APTT and a normal coagulometric APTT in plasma of a subject with a mild FXII deficiency (FXII 53% of normal). In terms of APTT ratios, the chromo-

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**Figure 3.** Correlation between chromogenic and coagulometric APTT in plasma of 44 healthy individuals (O) and 50 patients treated with oral anticoagulants (*) \( r = 0.48 \) and 0.71, respectively.

The lines denote the upper limits of the reference intervals.

**Figure 4.** Correlation between chromogenic and coagulometric APTT ratio (APTT patient's plasma/APTT normal pooled plasma) in plasma of patients with a mild congenital intrinsic clotting factor deficiency \( n = 24 \), r = 0.50.

The line denotes the upper limit of the reference intervals. (O) FVIII-deficient, (O) FXII-deficient, and (*) FXI-deficient plasma.

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genic APTT was slightly less sensitive to an FVIII deficiency (hemophilia A or von Willebrand's disease) than was the coagulometric APTT, although no FVIII deficiency was missed by the chromogenic APTT (Spearman's $r = 0.74$).

Comparison of the chromogenic ($y$) and coagulometric ($x$) APTT ratio in 18 patients with liver disease (mostly liver cirrhosis) showed a good correlation: $r = 0.93$. The orthogonal regression relationship was $y = 0.85x + 0.26$ \((S_y = 0.17)\). In terms of APTT ratios, the chromogenic and coagulometric APTT showed comparable results for the complex coagulation disorders of liver diseases (most of these patients showed a combination of FIX, FXI, and FXII deficiencies plus high values for FVIII). In two patients' plasma samples the chromogenic APTT ratio was increased but the coagulometric APTT ratio was still within the normal range. These patients appeared to have a mild FXII deficiency (FXII 54% and 62% of normal).

Finally, we compared the chromogenic APTT ratio with the coagulometric APTT ratio in plasma of 13 patients with disseminated intravascular coagulation (Figure 5) and found a poor correlation. In three samples the chromogenic APTT ratio was increased, with the coagulometric APTT ratio still in the normal range or only slightly increased; these plasma samples also showed a mild FXII deficiency. In contrast, two samples showed an increased coagulometric APTT ratio with a normal chromogenic APTT ratio: besides high concentrations of FDP, one sample contained a high fibrinogen concentration and the other a low concentration: 10.8 and 0.9 g/L, respectively (reference interval 1.7–4.0 g/L).

**Discussion**

The introduction of synthetic chromogenic substrates specific for coagulation factors has provided the possibility of developing new coagulation assays. Numerous chromogenic assays for specific coagulation factors are already available and have found clinical use. The development of chromogenic methods for the overall coagulation screening tests PT and APTT has been more cumbersome, but recent progress has been made, especially for the PT assay.

Behring has introduced a chromogenic APTT test, in which the traditional pre-incubation step required for optimum contact activation is omitted. The detection of thrombin generation with a chromogenic substrate rather than fibrin formation and with a much lower concentration of clotting factors represents a significant change in the APTT method. The biochemical conditions of the assay are therefore quite different from those found in conventional two-step APTT assays, which can produce different results in patients with hemostatic defects.

In this study we found the precision of the one-step chromogenic APTT to be comparable with results we obtained in previous experiments with a semiautomated two-step coagulometric APTT (KC-10A, Amelung, Limes, F.R.G.; Biometric B10, Sarstedt, Rommelshöfer, F.R.G.). Equivalent reproducibility was found by Dati et al. (11), using the same chromogenic APTT, whereas Duncan et al. (20), who used another chromogenic APTT in their study, reported a lower reproducibility. Furthermore, we found good repeatability for determinations of the intrinsic clotting factor by using the chromogenic APTT; this also compared well with that of a semiautomated coagulometric method.

The paradoxical decrease of the chromogenic APTT after mixing normal pooled plasma with increasing amounts of plasma deficient in FIX, FXI, FXII, or HMWK was observed only when immunoabsorbed deficient plasma samples were used. During the immunoabsorption procedure, perhaps contact activation of the plasma occurs, producing activation products that may shorten the chromogenic APTT in the absence of a pre-incubation period. Using specific monoclonal antibodies, we detected high concentrations of complexes of FXIIa-C1 esterase inhibitor and kallikrein-C1 esterase inhibitor in the immunoabsorbed deficient plasmas, whereas only very low concentrations of these complexes could be demonstrated in the congenital deficient plasmas. This phenomenon might also be expected to influence the precision of determinations of the intrinsic coagulation factors when these immunoabsorbed deficient plasmas were used. Probably because the clotting factor tests use a lower clotting factor concentration (normal plasma and patients' plasmas are diluted in the coagulation factor assay), no such influence can be noticed in the clotting factor assays (Table 1). Using
congenital deficient plasma in in vitro sensitivity studies gave satisfactory results for the chromogenic APTT assay, although the APTT method including Actin seemed to be more sensitive to deficiency of FVIII and FXI. We could not confirm the high sensitivities reported by Dati et al. (11). However, in their study the upper limit of the reference range is lower, which contributes to an apparently higher sensitivity.

Our studies with patients demonstrate the usefulness of the one-step chromogenic APTT as a screening test for intrinsic factor deficiency. Although the correlation between the two APTTs was not impressive, the chromogenic APTT was well able to discriminate a normal from a congenital factor-deficient patient, which is the main objective of a screening test. In addition, the chromogenic APTT is sensitive to the acquired hemostatic defects of the intrinsic system in patients with liver disease. One sample from the congenital deficient patient group and two samples from the patient group with liver disease demonstrated a prolonged chromogenic APTT and a normal coagulometric APTT. All three samples were obtained from patients with a mild FXII deficiency, emphasizing the sensitivity of chromogenic APTT to an FXII deficiency. Patients with disseminated intravascular coagulation yielded discrepant results when the two APTT methods were compared—possibly because of FXII deficiency or abnormal fibrinogen contents and (or) high concentrations of FDPs.

Duncan et al. (20) reported that the chromogenic APTT appeared to be sensitive to an FVII deficiency. We could not confirm these results, because congenital FVII-deficient plasma showed a normal chromogenic APTT. In addition to a different APTT reagent and a different test procedure, Duncan et al. used a higher sample dilution in the activation mixture, which may have enhanced interaction between the intrinsic and extrinsic coagulation pathways.

Another field in which APTT is used extensively, besides screening for intrinsic factor deficiency, is in monitoring patients receiving heparin therapy. In assessing the suitability of an APTT method for this, one commonly evaluates its performance by assaying normal (pooled) plasma heparinized in vitro. We found that the two APTT methods exhibited a similar sensitivity to in vitro heparinized plasma. However, the sensitivity of the chromogenic APTT to heparin in vivo appeared to be slightly less than that of the coagulometric assay, although the former seems adequate for clinical practice. The correlation between the two APTTs was moderate (Spearman's $r = 0.79$), and both the chromogenic and the coagulometric APTT correlated poorly with the heparin concentration. As has been observed, different APTT reagents vary widely in their sensitivity to heparin (18, 21–23); furthermore, the in vivo sensitivity of the APTT to heparin differs from the in vitro sensitivity (24–26). Moreover, a wide between-subject variability in sensitivity to heparin in vivo for a given APTT reagent has been reported (18, 27).

In conclusion, we find that this chromogenic equivalent of the APTT is promising as a routine test for the clinical laboratory. It appears to be a simple, reproducible assay that is easily adaptable to automated instruments. The sample volumes required are much smaller than for conventional APTT assays. The sensitivity to clinically relevant hemostatic defects involving the intrinsic coagulation system and to heparin in vivo is satisfactory, but in general slightly lower than the coagulometric APTT tested. Further correlation studies between the chromogenic APTT and coagulometric APTT might aid in defining a therapeutic range for heparin monitoring by the chromogenic APTT. As stated recently, this should preferably be assessed by using in vivo rather than in vitro heparinized patients' samples (28). The chromogenic assay is insensitive to hemostatic anomalies involving the extrinsic coagulation pathway. Like all chromogenic screening assays, it is relatively insensitive to anomalies in the fibrinogen–fibrin conversion. Our findings suggest that this one-step APTT may be sensitive to activated products of the contact system; the implications of this for clinical practice are not yet clear and need further evaluation. Finally, additional studies are required to establish the influence of inhibitors such as the lupus anticoagulant and specific factor inhibitors.

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References
Changes in Oxygen Measurements When Whole Blood Is Stored in Iced Plastic or Glass Syringes

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Tonometryed whole-blood and plasma specimens were tested in plastic and glass syringes to determine whether clinically significant changes in gas tensions occur during sample storage. When whole blood was tonometryed with 60 and 100 mL/L (6% and 10%) oxygen and then stored for 30 min in iced plastic syringes, the $\rho_{O_2}$ of the samples remained stable (mean change = +0.4 and +0.8 mmHg, respectively). However, for 140 mL/L (14%) oxygen tonometry, the $\rho_{O_2}$ increased significantly (mean change = +8.4 mmHg; $P <0.0001$). When tonometryed plasma was stored in iced plastic syringes, the $\rho_{O_2}$ increased progressively at all three concentrations, with the smallest change occurring at 140 mL/L (mean change = +12.6 mmHg) and the greatest at 60 mL/L oxygen (mean change = +20.9 mmHg). In contrast, when iced glass syringes were used for storing plasma or whole blood, no clinically significant changes in $\rho_{O_2}$ were found at any of the tonometryed oxygen values for 60 min. When whole blood was stored in plastic syringes at ambient temperature for 30 min, again no clinically significant changes in $\rho_{O_2}$ were found at these tonometry conditions. Apparently, some blood gas samples stored in iced plastic syringes may yield clinically significant errors in oxygen tension.

Additional Keyphrases: blood gases · analytical error · sample handling

All-glass syringes have proven effective in maintaining the integrity of blood gas values during the pre-analytical storage period (1). Many studies have attempted to validate plastic syringes as satisfactory substitutes for all-glass syringes (2–7). However, the conclusions have been conflicting and controversial, perhaps because of differences in experimental design and because of failures to (a) use clinically relevant syringe sizes and sample volumes (2–6); (b) test the effects of sample storage in ice water (3, 5, 6); (c) store and test gases dissolved in whole blood or plasma (4); or (d) take into consideration the initial oxygen concentrations of the stored samples (3, 5, 7).

Therefore, we designed an experiment incorporating these important factors to investigate what mechanism might cause analyte changes in samples stored in iced plastic syringes. Blood and plasma specimens tonometryed to various extents were tested because the initial oxygen concentration of the sample may influence the amount of the subsequent change in measured $\rho_{O_2}$.

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