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# Evaluation of Activated Partial Thromboplastin Time (aPTT) Reagents for Application in Biomedical Diagnostic Device Development

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Evaluation of activated partial thromboplastin time (aPTT) reagents for application in biomedical diagnostic device development

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aPTT, clotting time, thrombin generation assay, stability, heparin sensitivity.

SUMMARY

The most commonly used test for monitoring heparin therapy is the activated partial thromboplastin time (aPTT). The performance of available aPTT reagents varies significantly. The aim of this study was to assess the suitability of dried aPTT reagents for the purpose of monitoring unfractionated heparin dose-response in diagnostic devices. Ten reagents were analysed in terms of their performance in liquid and in dried form after 24 h and 14 days. The ability to reduce the natural plasma clotting time (CT) and their sensitivity to heparin was assessed. The thrombin generation assay was the method of choice. All reagents resulted in significant reductions in CT. Liquids returned more rapid CTs in comparison to dried reagents. Most reagents were more sensitive to heparin in dried, rather than in liquid form. Dried reagents based on kaolin as a surface activator were notably more effective in achieving rapid CT, while reagents composed of silica and synthetic phospholipids were the most sensitive to heparin. Identification of the most suitable aPTT assay reagent for incorporation into a diagnostic device platform was achieved based on dried reagent stability and responsiveness to heparin.

INTRODUCTION

There has been growing interest in the development of miniaturized, point-of-care diagnostic devices (Khandurina and Guttman, 2002). There is an increasing requirement for reliable point-of-care devices for monitoring the effect of drugs which regulate blood coagulation (Khan, 2009). This in turn leads to an increased demand for highly concentrated and optimized formulations of active ingredients capable of inducing a rapid clotting response in a low volume test sample. Since the first application of partial thromboplastin time (aPTT) for monitoring anticoagulant therapy (Struver and Bittner, 1962), it has become the most popular test for heparin dose monitoring (Gawoski et al., 1987) and is regarded as the most reliable and commonly used test (Kitchen et al., 1996) for heparin dosage adjustment in long term therapy and pre-thrombotic monitoring (Bowers and Ferguson, 1993). There have been several automated devices developed to monitor aPTT, e.g. the CoaguCheck® Pro. In general, such devices contain dry formulations that selectively induce the clotting process. The accuracy and reliability of the results are highly influenced by the quality of the dried chemistry. There are a growing number of ready-to-use aPTT reagent kits available in liquid or lyophilized forms. A number of studies have identified that aPTT reagents vary significantly in their responsiveness to heparin (Kitchen et al., 1999, Banez et al., 1980). Different aPTT reagents often return different aPTT values with normal patient plasmas. There is an urgent need for the standardization of aPTT-based monitoring systems for heparin therapy in clinical settings (Poller et al., 1989, Kitchen et al., 1996). The varying responsiveness of aPTT reagents is dependent upon the composition of particular constituents of the formulation. These contain animal or plant extracts as sources of phospholipids (partial thromboplastin) combined with a surface activator such as kaolin, celite, silica, ellagic acid, dextran sulphate or carrageenans. Negatively charged substances supported by phospholipids bring about the surface-dependent activation of factor XII which is the first step of the contact activation pathway leading to the eventual formation of the insoluble fibrin clot (Griffin and Cochrane, 1979). Both, the source and the concentration of phospholipids have been highlighted as important factors in assay behaviour, e.g. a decreased phospholipid concentration can lead to an increased heparin sensitivity of the reagent (Ts'ao et al., 1998, Kitchen et al., 1999). Stevenson et al. documented that the lipid phase concentration may differ by 300 times between some commercially available aPTT reagents (Stevenson et al., 1986). Advances in technology have led to the development of recombinant materials for use in coagulation assays. Such defined and reproducible materials might help to control the variation that exists within aPTT activated clotting response (Barrowcliffe and Gray, 1981, Triplett, 1982). Several reports provide a detailed characterization of aPTT reagents (Stevenson et al., 1991, Kitchen et al., 1999, Martin et al., 1992). These are extremely informative sources of data on aPTT reagent composition and performance in clinical and laboratory settings. The differences in their sensitivity to heparin and lupus antibodies have been shown (Eby, 1997, Manzato et al., 1998). However, there is very little data regarding the use of available aPTT formulations for the purpose of diagnostic device development. Due to a wide variety of aPTT products with different formulations and physical characteristics, an assessment of the aPTT performance in dried form and its stability upon prolonged storage time was needed. Evaluation of the dried-surface reagents would allow selection of the most suitable activators to be incorporated into a coagulation monitoring assay. The effect of resolubilization and drying on their activity is of particular relevance.

The thrombin generation assay (TGA) was chosen as a tool for the comparison of the ability of aPTT reagents to reduce natural plasma clotting time (CT) by measuring the rate of thrombin generation (van Veen et al., 2008). This study includes a comparison of a variety of aPTT formulations in (i) liquid, (ii) 24 h dried and (iii) 14 days dried forms. These were analysed in terms of their ability to reduce plasma CT, stability upon prolonged exposure to ambient conditions and responsiveness to heparin.

MATERIALS AND METHODS

**Materials**

Ten aPTT reagents were studied (Table 1). All assays were performed using Hemosil plasma (0020003710, Instrumentation Laboratory, Italy/USA). The colorimetric thrombin substrate was H-D-Phenylalanyl-L-pipecolyl-L-arginine-p-nitroanilinedihydrochloride (S-2238, Chromogenix, Italy/USA) and was prepared by dilution 1:4 with Tris.HCl pH 8.3. Calcium chloride ($CaCl_2$) (100989) was from BioData (Netherlands/USA). Heparin sodium salt was from Sigma, Germany/USA (H0777-100KU), Greiner BioOne 96 well microassay plates (655096, Greiner BioOne, Germany) were used for all assays. All water was 18 MΩ or greater.



**Thrombin Generation Assay**

Assays were carried out using 96-well polystyrene microassay plates. Each test well (n = 5) contained 50 µL aPTT reagent, 50 µL plasma, 50 µL colorimetric substrate and 50 µL 25 mM $CaCl_2$. aPTT reagent was pre-incubated with plasma according to manufacturer recommendations. Subsequently, colorimetric substrate and $CaCl_2$ were added. Measurement was started immediately after $CaCl_2$ addition. The amount of generated thrombin was determined colorimetrically by measuring the release of *p*-nitroaniline (pNA) from the chromogenic substrate on a Tecan Infinite M200 (Tecan Group Ltd., Switzerland) at 405 nm with measurements made every 30 s for 1 h. TGA assays yielded absorbance profiles which related to the generation of thrombin following activation of the intrinsic clotting cascade. A positive thrombin control was also included in every assay where aPTT reagent was replaced with 50 µL of thrombin at a concentration of 3 U/mL (Sigma-Aldrich Inc.). aPTT reagents were analysed in both liquid and dried forms, with the latter being evaluated at 24 h and 14 days following drying. For drying, 50 µL of aPTT reagent were pipetted into the 96 well plates and left to dry under ambient laboratory conditions. The dried reagent was reconstituted in 50 µL of water prior to analysis. Thrombin generation profiles for dried reagents at 24 h and 14 days were compared to those for liquid controls.

Reagents were analysed in terms of their sensitivity to heparin using spiked plasma samples. It has been shown that the response from *ex vivo* samples from patients on heparin therapy differs from *in vitro* plasma samples spiked with heparin (Jespersen et al., 1999, Van den Besselaar et al., 1990). However, heparin-spiked samples were used herein for the purpose of a performance comparison between reagents and not for the aPTT clinical reference standardization. Control plasma was spiked with heparin so that the final concentration in plasma was between 0 and 2 U/mL.

**Data Analysis**

The activity of the aPTT reagents was determined by their ability to reduce the natural plasma CT. Three methods of CT calculation were employed which were (i) the length of the lag time (LT), which was taken as the time prior to the occurrence of the thrombin burst (observed as a rapid increase in the measured absorbance) followed by a propagation phase (Wolberg, 2007), (ii) the area under the curve (AUC) and (iii) the time-to-peak (TTP). AUC was calculated from both raw (absorbance vs time) and differentiated (dAbs vs time) data. This reflects the total time for which thrombin exerts its enzymatic activity and is related to the endogenous thrombin potential. TTP was taken as the time point where the response profile reached a plateau and the increase in absorbance ceased. This is related to initiation of thrombin production and propagation and corresponds to the time required for the available substrate to be utilized (Wolberg, 2007). Analysis of these characteristic response profiles led to the determination of CT based on LT. CT values were related to heparin concentration and the resulting correlations provided useful information about the dry and liquid reagent sensitivity to heparin. Inter- and intra-assay variation (CV) was also determined.

RESULTS

**Clotting time extraction.** Fig. 1 shows typical thrombin generation profiles for human plasma samples with and without activation by Cephalinex aPTT reagent. The thrombin generation profile of the blank control showed a LT greater than 1500 s, a rapid increase in absorbance and a plateau at about 2500 s. In most assays, corn trypsin inhibitor is added to prevent controls from clotting. However, as a control to assess the level of inter-assay variability, non-activated controls were used (Lo et al., 2005). The average control plasma LT for all assays was found to be 1943 s with a %CV of 15.6%. Addition of liquid Cephalinex aPTT reagent resulted in the rapid onset of the thrombin burst resulting in an indeterminate LT. The linear fluorescence response was reached at about 150 s, with the plateau being reached at approx. 200 s. In comparison, when reagent was dried, an increase in the LT of approx. 100 s was noted, which suggests that, for this reagent at least, drying did result in increased LT. However, there was little change in LT between 24 h and 14 days storage (LTs of around 300 s).

**Fig. 1.**

Several methods were employed to correlate the TGA output with the change in CT following incubation with heparin as shown for aPTT-SP in Fig. 2. Heparin plays a role in the positive-feedback inhibition of thrombin (Beguin et al., 1988) and was used here to obtain prolonged clotting times. LT and TTP calculations resulted in similar correlations between CT values and heparin dosage. LT values were predictably lower than the TTP values by the difference in rise time for the thrombin burst. Both these parameters were prolonged with increased heparin concentration. The correlation was close to linear for both methods with $R^2 \geq 0.986$. Calculation of the area under the curve

(AUC) was not found to be a useful tool in CT determination. No correlation between calculated area values and heparin dose was found. LT was thus chosen as the most convenient and reliable way of determining the clotting time (CT) values and all further expressions of CT were based on this methodology.

**Fig. 2.**

**Comparison of aPTT reagent activities in liquid and dried forms**

It has been established in many studies that commercially available aPTT reagents are effective for plasma CT reduction in their liquid form. However, an important parameter from the point of view of bioassay development is that reagents need to maintain their pro-coagulant properties upon long-term storage in a dehydrated state. The performance of aPTT reagents in their liquid and 24 h / 14 day dried forms was assessed by TGA according to their CT values (Fig. 3).

**Fig. 3.**

As expected, the performance of all aPTT reagents in their liquid forms was excellent. The liquid state was recommended by manufacturers to be used for plasma CT assays. CT values between 41 ±12.8 s for C.K. Prest 2 and 161 ±23.8 s for SynthASIL were obtained. In addition to comparisons of reagent activity in their liquid forms, the stability of the ready-to-use formulations was also assessed following drying and storage for 24 h and 14 days. Short term storage (24 h) did affect the activity of most reagents. CTs obtained for Platelin LS after 24 h were significantly prolonged, from 95 ±21.3 s in liquid to 468 ±15.9 s. CTs of dried reagents were at least twice as long as that for the equivalent liquid reagent: Alexin (85 ±7.1 s to 180 ±52.3 s), Cephalinex (110

±9.8 s to 288 ±55 s), C.K. Prest 2 (41 ±12.8 s to 96 ±15.9 s), aPTT-P (72 ±11.3 s to 218 ±19.3 s) and aPTT-SA (83 ±1.4 s to 184 ±24.2 s) for liquid and 24 h dried CTs, respectively. The only exception was aPTT-SP which proved to be perfectly stable after 24 h yielding a shortened CT over the liquid form by approx. 10 s.

In order to establish if the ability of the aPTT to activate clotting continued to deteriorate with prolonged storage, additional tests were performed after 14 days. For all reagents, the CT values gradually increased with one exception; Cephalinex returned a CT of around 280 s for 24 h and 14 day measurements. The ability of Platelin LS and Alexin to reduce the plasma CT decreased dramatically to 1566 ±415.7 s and 850 ±130.7 s for 14 days Platelin LS and Alexin, respectively. The effect of reagent deterioration in the form of increased CT was expected to correlate with prolonged storage. Such reagents would not be considered as suitable candidates for a dried matrix of coagulation activators incorporated into a clot monitoring device. The addition of stabilizing buffers, preservatives or special storage conditions could be beneficial in the improvement of stability. However, the aim was to rapidly establish the activity of the dried ready-to-use reagents in order to select the most stable formulations. The effect of a diminished CT reduction was not as strongly manifest for the other eight reagents as it was for Platelin LS and Alexin. CTs were between 200 ±17.3 s for Dapttin to 438 ±135.2 s for aPTT-P.

The ideal dried formulation not only had to reduce the plasma CT efficiently and be stable over a prolonged period of storage, but should also generate reproducible results and so inter- and intra- variability was a factor of importance. The within-run %CV was maintained at ≤ 15% for all tested reagents (n = 5), while significant differences in the inter-assay variability (n = 3) was noticed. It was observed that reagents that were more affected by the storage time and conditions, resulting in a prolonged CT (in comparison to a liquid control) were also the least precise (highest %CV for between-assay variability). These were Platelin LS, Alexin, aPTT-P and aPTT-SA which yielded CTs of 1566 s (26.5%), 850 s (15.4%), 438 s (30.9%) and 314 s (40.5%) respectively. The remaining six reagents resulted in CTs < 300 s and %CV of less than 15%.



**Heparin sensitivity**

A striking variation in aPTT reagent sensitivity to thrombin inhibitors such as heparin has been documented (Greaves, 2004). Performance of aPTT reagents is highly dependent on the source and content of phospholipids as well as on the type and concentration of surface activator that is supposed to serve a large surface area for stimulation of kallikrein-like activity and initiation of coagulation. Several studies have reported the association of aPTT reagent composition with variations in clotting efficacy (Smeets et al., 1996, Neuenschwander et al., 1995) and responsiveness to anticoagulant dosage (Ip et al., 2001, Kitchen et al., 1996). Herein, the effect of the drying process and storage of the aPTT reagents was also evaluated for its effect on the response to heparin in the TGA. Such data is of great importance for the development of miniaturized systems for anticoagulant therapy monitoring.

The effect of heparin addition (0 – 2 U/mL) on plasma CT triggered by all ten aPTT reagents in their liquid and dried forms was evaluated by TGA. Reagent sensitivity to heparin was assessed on the basis of the derived calibration curves. For example, the results for aPTT-P are shown in Fig. 4. The correlation between plasma CT and heparin concentration was found to be linear for liquid and dried reagent with $R^2 > 0.98$. However, the sensitivity to heparin was significantly different as evidenced by changes in slope. The 14 day form was found to be the most responsive to anticoagulant in which the CT increased by 392 s upon addition of heparin at 2 U/mL. In comparison, CT values for liquid and 24 h dried reagent were prolonged by 127 s and 150 s, respectively. The slope values illustrate the difference in heparin sensitivity: 62.2, 72.8 and 193.6 s.mL.$U^{-1}$ for liquid, 24 h and 14 days dried, respectively. However, it must also be taken into account that the base CT (0 U/mL heparin) was significantly increased due to drying from liquid (72 ±11 s) to 24 h (218 ±19 s) and 14 days (438 ±135 s), which suggested a significant deterioration in reagent activity. Therefore, regardless of higher heparin sensitivity, such a reagent would not be suitable. Furthermore, this reagent at 14 days yielded elevated %CV in comparison to the two other tested forms (31% in comparison to 16% and 12% for liquid and 24 h dried reagent, respectively).

**<u>Fig. 4.</u>**

Similar analyses were performed for the other reagents, the results of which are summarised in Table 2.  Addition of heparin resulted in the prolongation of CT for all tested reagents. Some of these were significantly affected by the presence of heparin in the sample, while others were less sensitive to its effect. The values obtained for liquid reagents increased from 41 – 161 s for 0 U/mL heparin to 102 – 286 s, 130 – 348 s, 139 – 405 s and 199 – 810 s for 0.5, 0.75, 1 and 2 U/mL of heparin, respectively. CTs obtained for 24 h dried reagent changed from 88 – 468 s to 138 – 602 s, 208 – 737 s, 186 – 880 s and 258 – 946 s with an increased heparin concentration. 14 day dried reagents returned an increase in CT from 200 – 1566 s to 256 – 1517 s, 262 – 1439 s, 280 – 1705 s and 369 s – > 1 h with increasing heparin concentrations, respectively. Although the issue of an increased base CT (0 U/mL heparin) occurred for all 14 day dried reagents, this form was generally the most responsive to heparin. The exceptions were Cephalinex, Dapttin and Alexin-HS for which the liquid form was the most sensitive to heparin and Platelin LS which was significantly affected by the drying process and 14 days of ambient storage resulted in the near complete loss of its potential to reduce plasma CT; CT values obtained for plasma with 0 – 1 U/mL heparin were 1566 – 1640 s as compared to 1943 s for non-activated plasma. Generally, a trend of prolonged CT with increased heparin concentration was observed. However, there was great variation in the CT values for different aPTT reagents which suggested significant differences in reagent quality.

The slope values in Table 2 indicate the differences in heparin sensitivity of the tested reagents both in liquid and dried forms. It should be noted that the slope values obtained for 14 day dried Platelin LS, Alexin and C.K. Prest 2 were quite high, but since no CT was obtained for 2 U/mL heparin and the correlation was based on only four data points, these reagents were excluded from the comparison of slope values for the 14 day dried reagents. SynthASIL was shown to be by far the best performing in this respect, yielding the highest slope values of 327.5, 312.1 and 443.1 s.mL.U$^{-1}$ for liquid, 24 h and 14 day dried forms, respectively. The non-heparinized plasma CTs of 161 ±24, 194 ±54 and 293 ±43 s were prolonged to 810 ±36, 806 ±35 and 1170 ±94 s, respectively, upon addition of 2 U/mL of heparin. Such large differences in CT values (at least 600 s) between non-heparinized and heparinized (2 U/mL) samples allowed good discrimination between samples of different heparin levels. The least responsive to heparin were aPTT-SA in liquid form (56.2 $s.mL.U^{-1}$), Alexin in 24 h dried form (48.7 $s.mL.U^{-1}$) and Cephalinex as 14 day dried reagent (56.5 $s.mL.U^{-1}$). These reagents returned insignificant CT differences when tested over a range of heparin concentrations. The use of these reagents in an anticoagulant monitoring device may result in the diminished discrimination between heparin doses. For the purposes of a reagent for use in a coagulation monitoring device, the activated CT is an extremely important factor determining the assay time. The shortest CTs were achieved for Dapttin with 95 ±1 s for non-heparinized plasma triggered with liquid reagent and 369 ±21 s for 2 U/mL heparin in plasma triggered with 14 day dried reagent. The longest CTs were obtained for 14 day dried C.K. Prest 2, Platelin LS and Alexin for which an addition of heparin at 2 U/mL resulted in an enormously prolonged CT with which it was not possible to extract a lag time.

## DISCUSSION

Very good linear correlations between CT and heparin concentration were found for nine out of the ten tested reagents within the heparin range of 0 – 2 U/mL ($R^2 \geq 0.83$). Platelin LS was the only reagent on which the drying process had a dramatic negative impact resulting in significant loss of activity. High variation and no correlation with increased heparin concentrations were also found for the 14 day dried samples ($R^2 = 0.02$). Even though most of the reagents tested responded to addition of heparin by returning prolonged CTs, the deterioration of control clotting values (base CT) also had to be taken into account when selecting a stable, dry formulation which was responsive to heparin. Among several methodological factors influencing the aPTT performance, the reagent composition is one of the most commonly cited (Manzato et al., 1998, Shetty et al., 2003, Wojtkowski et al., 1999). The available surface area of the negatively charged surface activators might be reduced due to dehydration following drying. The surface activation may be influenced by the type of activator used. Drying processes may have influenced the orientation and distribution of phospholipids. Some classes of phospholipids are of particular importance, and depending on their ratios and the total concentration of lipids, clotting can be promoted or inhibited (Slater et al., 1980, Comfurius et al., 1994). Liquid reagents containing phospholipids derived from rabbit brain cephalin (C.K. Prest 2, aPTT-P, aPTT-SA and Alexin) returned the shortest CTs varying between 41 – 85 s. However, their ability to reduce plasma CT was significantly impacted by the drying process. The chicken and porcine-derived phospholipids used in Platelin LS gave an extremely prolonged CT in dried form (up to 1566 s for 14 days dried reagent). APTT-SP (silica and synthetic phospholipids) was the only reagent for which the absolute CT did not increase after 24 h following drying. However, prolonged storage under ambient conditions did result in the reagent's eventual deterioration. Another reagent which performed reasonably well was Cephalinex (containing silica activator and rabbit brain phospholipids). Despite the fact that the 24 h drying did influence the reagent's ability to shorten plasma CT, the long term storage did not seem to affect its activity. However, Cephalinex was shown to be less sensitive to heparin than aPTT-SP in all three tested forms.

It has been also shown that the type and concentration of surface activator plays a major role in the effectiveness of aPTT reagents (Marlar et al., 1984). Reagents based on kaolin, (C.K. Prest 2) or kaolin/sulfatides (Dapttin) were shown to return rapid CTs in all three tested forms, not exceeding 100 s for liquid, 130 s for 24 h dried and 300 s for 14 day dried forms. They were always among the five reagents with the shortest CTs. The combination of traditionally-used kaolin and rabbit brain cephalin extract (C.K. Prest 2) seemed to achieve rapid CTs. However, it should be taken into account that the use of insoluble particulates such as kaolin or silica for automated devices employing photo-optical detection may be problematic. The use of a soluble ellagic acid or a mixture of low concentration insoluble activators such as kaolin and sulfatides i.e. Dapttin (Moritz and Lang, 1995) may be a solution for devices based on optical detection systems.

Although the reasons for the variable heparin sensitivity of the tested aPTT reagents have not been determined, the nature of surface activator and lipid composition seem to play a major role. The relationship between aPTT reagent lipid composition and heparin sensitivity was thoroughly investigated by Kitchen et al. (1999). Van den Besselaar et al. (1997) described a method of aPTT formulation preparation consisting of colloidal silica and synthetic phospholipids and suggested that the synthetic reagents should form a foundation for aPTT standardization of heparin therapeutic control. In fact, in our study, synthetic phospholipid-based reagents (SynthASIL and aPTT-SP) performed extremely well in terms of both heparin response and dried reagent stability. Additional benefits of such homogeneous materials based on synthetic phospholipids includes minimized batch-to-batch variability which leads to improvements in test CVs (Okuda and Yamamoto, 2004). Both aPTT-SP and SynthASIL which are both based on synthetic phospholipids and silica and are both manufactured by Hemosil, were identified as promising candidates for incorporation into point of care diagnostic device platforms as dried reagents.

TABLES

Table 1. Panel of aPTT reagents including the manufacturers and the composition*.

| Reagent | *Producer* | Surface Activator | Source of phospholipids |
|---|---|---|---|
| **APTT-SP** | *Hemosil* | Colloidal silica | Synthetic |
| **Cephalinex** | *BioData* | Microsilica | Rabbit brain |
| **SynthASIL** | *Hemosil* | Colloidal silica | Synthetic |
| **Platelin LS** | *BioMerieux* | Micronized silica | Porcine + chicken |
| **C. K. Prest 2** | *Diagnostica Stago* | Kaolin | Rabbit brain |
| **Dapttin** | *Technoclone* | Synthetic kaolin+sulfatides | Unknown |
| **Alexin** | *AMAX / Trinity Biotech* | Ellagic acid | Rabbit brain |
| **Alexin-HS** | *AMAX / Trinity Biotech* | Ellagic acid | Rabbit brain + soy |
| **APTT-SA** | *Helena BioSciences* | Ellagic acid | Rabbit brain |
| **APTT-P** | *Biopool* | Magnesium-aluminium-silicate | Rabbit brain |

*Data is according to available manufacturer's information.

Table 2. aPTT reagents in liquid and dried forms (24 h and 14 days) characterized in terms of their heparin dose sensitivity (slope), normal derived CT (intercept), linear correlation with heparin from 0 to 2 U/ml ($R^2$), maximum standard deviation (SD) and maximum percentage coefficient of variation (CV).

| Reagent | Form | Slope [$s.mL.U^{-1}$] | Intercept [s] | $R^2$ | Max. SD [s] | Max. CV [%] |
|---|---|---|---|---|---|---|
| aPTT-SP | Liquid | 113.6 | 107.2 | 0.99 | 48 | 33 |
| | 24 h | 84.0 | 96.2 | 0.99 | 63 | 44 |
| | 14 days | 134.3 | 289.2 | 0.96 | 96 | 20 |
| Cephalinex | Liquid | 65.5 | 117.3 | 0.98 | 23 | 9 |
| | 24 h | 57.8 | 261.6 | 0.83 | 68 | 22 |
| | 14 days | 56.5 | 284.4 | 0.98 | 35 | 10 |
| C.K. Prest 2 | Liquid | 95.0 | 51.9 | 0.98 | 22 | 31 |
| | 24 h | 118.7 | 112.7 | 0.97 | 74 | 22 |
| | 14 days | 561.8 | 249.0 | 0.99 | 106 | 13 |
| SynthASIL | Liquid | 327.5 | 123.6 | 0.98 | 36 | 15 |
| | 24 h | 312.1 | 148.4 | 0.97 | 54 | 28 |
| | 14 days | 443.1 | 260.2 | 0.99 | 94 | 15 |
| Platelin LS | Liquid | 68.6 | 103.6 | 0.98 | 22 | 22 |
| | 24 h | 244.6 | 518.6 | 0.85 | 137 | 15 |
| | 14 days | (-53.2) | 1516 | 0.02 | 416 | 27 |
| aPTT-P | Liquid | 62.2 | 77.5 | 0.99 | 11 | 16 |
| | 24 h | 72.8 | 223.7 | 0.99 | 37 | 12 |
| | 14 days | 193.6 | 457.4 | 0.99 | 135 | 31 |
| aPTT-SA | Liquid | 56.2 | 103.7 | 0.90 | 51 | 25 |
| | 24 h | 83.8 | 196.0 | 0.98 | 84 | 29 |
| | 14 days | 131.1 | 345.8 | 0.95 | 237 | 49 |
| Dapttin | Liquid | 105.1 | 96.3 | 1.0 | 49 | 16 |
| | 24 h | 79.1 | 140.8 | 0.96 | 30 | 14 |
| | 14 days | 82.1 | 203.6 | 0.99 | 49 | 17 |
| Alexin | Liquid | 61.4 | 86.6 | 1.0 | 7 | 8 |
| | 24 h | 48.7 | 172.2 | 0.97 | 80 | 34 |
| | 14 days | 829.5 | 867.4 | 0.98 | 359 | 27 |
| Alexin-HS | Liquid | 114.5 | 138.9 | 0.99 | 35 | 27 |
| | 24 h | 83.3 | 118.8 | 0.95 | 49 | 14 |
| | 14 days | 91.7 | 263.1 | 0.98 | 55 | 12 |

FIGURE LEGENDS

Fig. 1. Thrombin generation assay clotting profiles of Cephalinex in liquid (circles) and dried for 24 h (triangles) and 14 days (squares). Blank control was non-activated plasma (diamonds) (n = 3).

Fig. 2. The relationship between heparin concentration and CT as calculated using lag time (LT) and time to peak (TTP) for aPTT-SP in liquid and dried after 24 h. The trend line parameters were found to be as follows: TTP 24h dried (circles) y = 102.32x + 255.83, $R^2$ = 0.986; TTP liquid (reversed triangles) y = 120.77x + 230.94, $R^2$ = 0.996; LT 24h dried (squares) y = 83.955x + 96.239, $R^2$ = 0.986 and LT liquid (diamonds) y = 116.18x + 88.045, $R^2$ = 0.997.

Fig. 3. CT values obtained from plasmas activated with aPTT reagent; liquid (white), dried for 24 h (grey) and dried for 14 days (black) (n = 5).

Fig. 4. Relationship between heparin concentration and obtained CT for aPTT-P (n = 5). Plasma spiked with heparin (0 – 2 U/mL) was triggered with liquid (circles), 24 h dried (reversed triangles) and 14 days dried (squares) reagent. The trend line parameters obtained were as follows: y = 62.182x + 77.545, $R^2$ = 0.9881, y = 72.818x +223.7, $R^2$ = 0.9883 and y = 193.64x + 457.41, $R^2$ = 0.9858 for liquid, 24 h and 14 day dried reagents, respectively.

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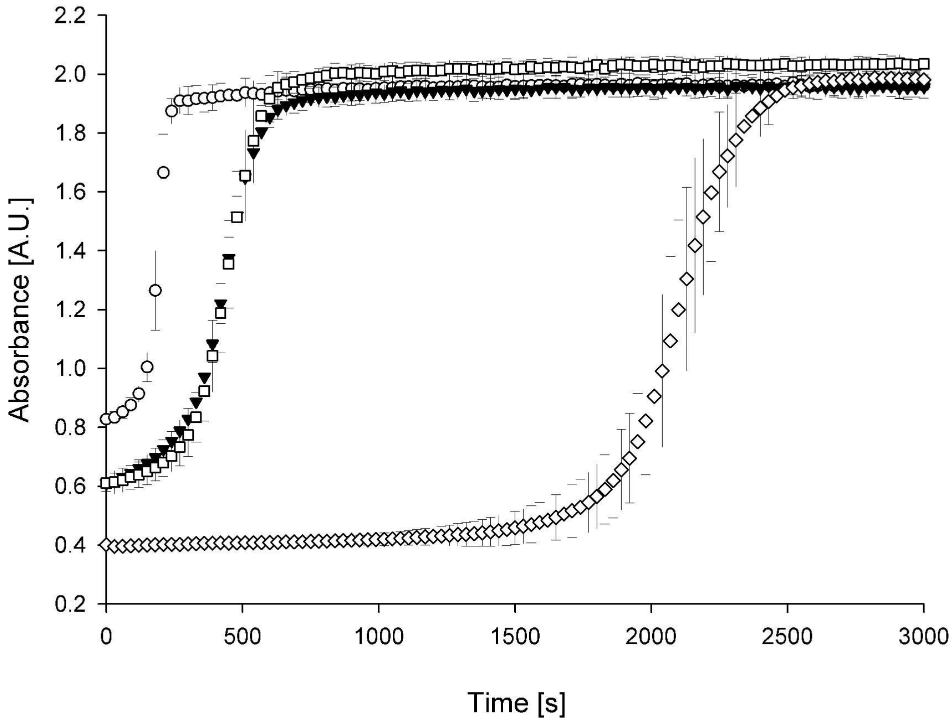


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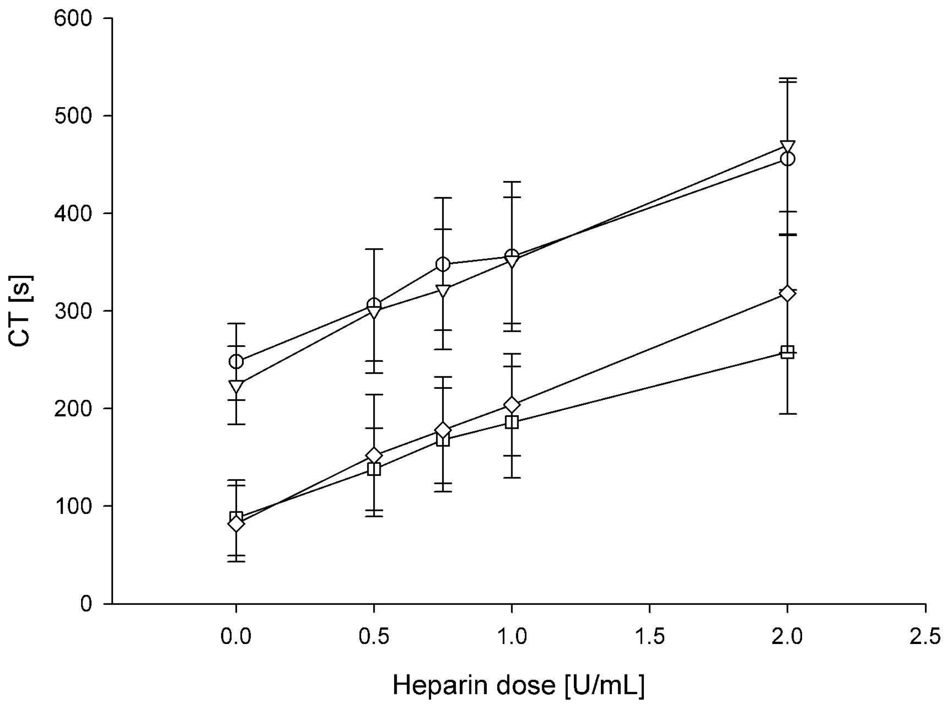


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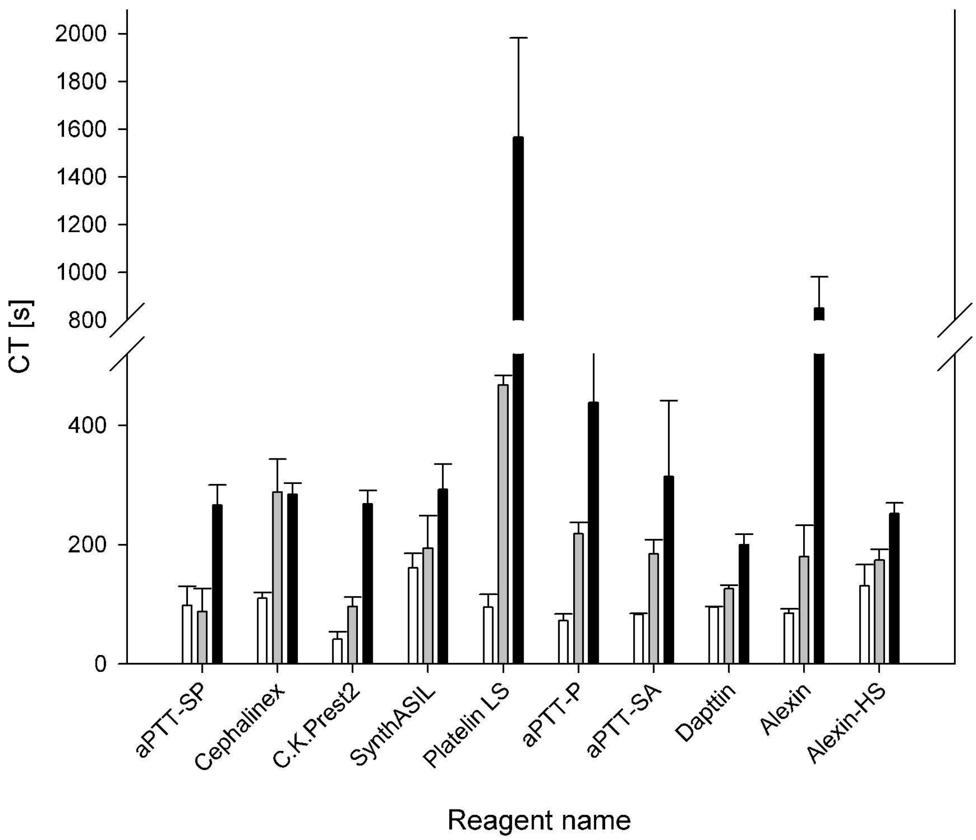


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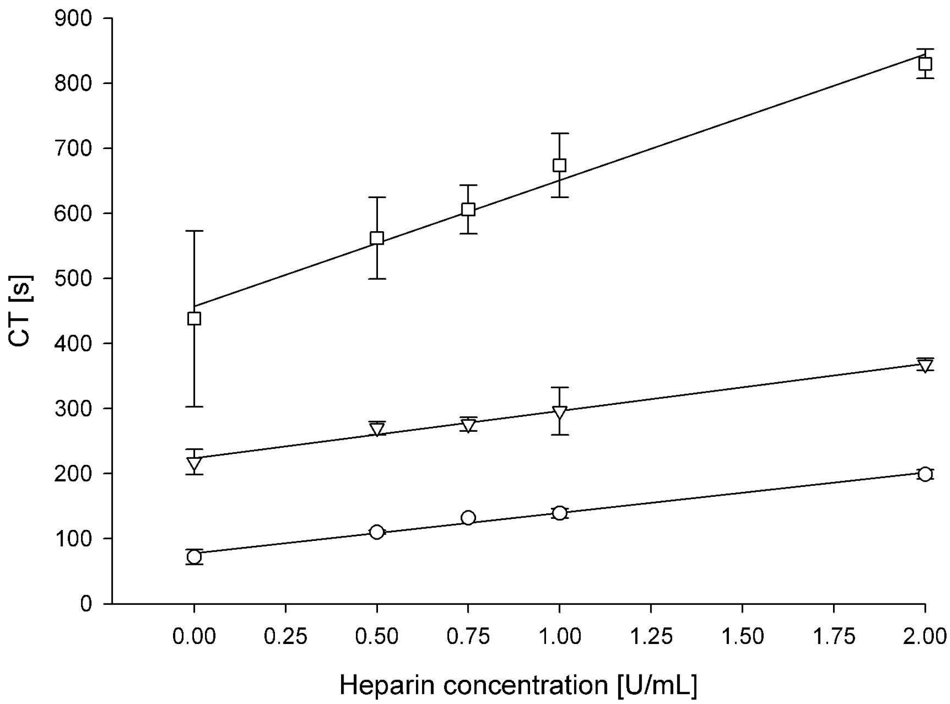


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