PF4-R, a Novel Heparin Neutralizing Reagent Suitable for Automated Coagulometers: Comparison with Heparinase in Routine Coagulation Tests

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Introduction

- A common source of pre-analytical error in the coagulation laboratory is heparin contamination of samples. Removal of the heparin effect is essential for obtaining accurate and reliable results in routine coagulation screening tests.
- Methods used for removing or neutralizing heparin *in vivo* or *in vitro* have included the use of agents such as protamine sulfate and hexadimethrine bromide (Polybrene®), the heparin-degrading enzyme heparinase (Hepzyme®), and extracorporeal heparin removal devices. However, each of these techniques has been associated with its own set of drawbacks, including treatment-induced anticoagulation or hypercoagulability[1,2].
- We have developed a novel, ready-to-use, fast-acting, stable platelet factor-4 based heparin neutralizing reagent (PF4-R)[3,4] for use in routine coagulation testing, that shows no interference with clot time results of non-heparinized plasma.

Objective

- To evaluate and compare PF4-R performance with heparinase in neutralizing unfractionated heparin (UFH) in routine coagulation tests (aPTT, PT, TT) and factor assays.

Methods

- Plasma samples used in this study include: CRYO*check*™ Pooled Normal Plasma (CCN), CRYO*check* Normal Donor Set (CCNS), CRYO*check* Heparin Control (CCH).
- Clinical samples were collected from patients with unexplained, elevated aPTT (Target population) and from patients medicated with UFH (Positive population).
- PF4-R treatment was performed as follows: Frozen PF4-R vials were thawed for 30 to 60 sec at room temperature before adding 0.5 mL of citrated plasma per vial. Vials were re-capped and gently inverted 3- to 5-times to mix. Samples were tested immediately or within 8 hours of treatment.
- Heparinase (Hepzyme) treatment was performed according to the manufacturer's instructions.
- Testing was performed on a STA-R Evolution® using STA®-PTT Automate, STA®-Neoplastine CI+, and STA®-Thrombin reagents according to the manufacturer's instructions.

Conclusion

- PF4-R is a novel, ready-to-use, stable reagent capable of fully neutralizing the anticoagulant activity of at least 2 IU/mL of UFH in plasma in routine clotting tests.
- PF4-R does not interfere with the clot time of non-heparinized plasma.
- PF4-R and heparinase treated patient samples showed a strong correlation in clot times in all tests.
- PF4-R and heparinase treatments gave similar aPTT and PT results, however the TT of heparinase treated plasma was prolonged by a mean of 6 sec relative to PF4-R. This bias was due to an interference by heparinase in the TT assay.

Results

Heparin Neutralization Capacity of PF4-R

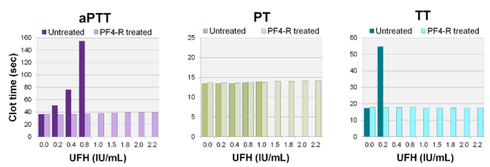

Figure 1. Clot times of untreated and PF4-R treated pooled normal plasma (CCN) samples containing up to 2.2 IU/mL UFH (heparin added *in vitro*).

Neutralization of Heparinized Normal Donor Samples

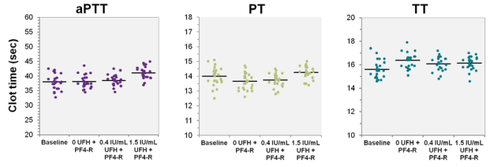

Figure 2. Clot times from 25 normal donor samples (CCNS) containing different amounts of UFH (heparin added *in vitro*) after treatment with PF4-R. Baseline shows the clot times of each donor sample without UFH and PF4-R.

Imprecision of Coagulation Tests with PF4-R Treated Samples

		aPTT		PT		TT	
Sample	PF4-R	S_r	S_T	S_r	S_T	S_r	S_T
CCN	-	0.30	0.57	0.08	0.19	0.25	0.78
CCN	+	0.24	0.71	0.14	0.31	0.25	0.56
CCN+0.4H	+	0.28	0.86	0.08	0.30	0.38	0.56
CCN+1.5H	+	0.24	0.80	0.08	0.36	0.22	0.60

Table 1. Repeatability (S_r) and within-laboratory precision (S_T) of untreated (-) and PF4-R treated (+) pooled normal plasma containing no UFH (CCN), 0.4 IU/mL UFH (CCN+0.4H) and 1.5 IU/mL UFH (CCN+1.5H). The largest standard deviation (in sec) from three PF4-R lots is shown.

Factor Assays

	FII (%)		FV (%)		FVII (%)		FVIII (%)		FIX (%)		FX (%)		FXI (%)		FXII (%)	
Sample	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
CCN	116.0	112.2	101.0	107.6	110.6	114.6	114.8	118.2	123.2	124.4	109.6	110.6	95.2	99.2	107.0	112.8
CCN+0.4H	112.4	112.4	99.2	106.0	98.8	107.4	65.6*	98.6	78.8*	129.4	103.4	110.4	65.4*	98.0	55.0*	113.2
CCN+1.5H	105.2	107.6	80.0	96.2	90.6	105.8	17.2*	91.4	14.2*	117.0	100.4	105.6	9.6*	89.2	10.2*	108.4

* Level is artificially low in untreated sample due to heparin interference with the factor assay.

Table 2. Factor activity levels in untreated (-) and PF4-R treated (+) pooled normal plasma containing no UFH (CCN), 0.4 IU/mL UFH (CCN+0.4H), and 1.5 IU/mL UFH (CCN+1.5H).

Long Term Storage Stability of PF4-R

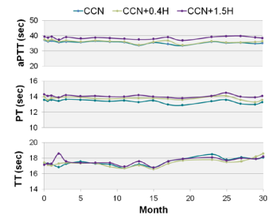

Figure 3. Clot times of pooled normal plasma containing no UFH (CCN), 0.4 IU/mL UFH (CCN+0.4H) and 1.5 IU/mL UFH (CCN+1.5H) after treatment with PF4-R that has been stored at -20°C for up to 30 months.

Comparison of PF4-R with Heparinase on Clinical Samples

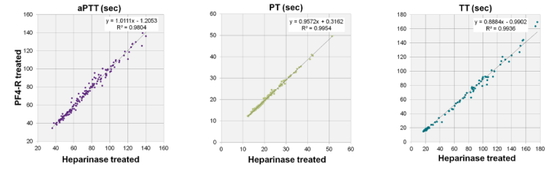

Figure 4. Correlation of clot times from a mixture of samples collected from patients with unexplained, elevated aPTT (Target population, N=100) and from patients medicated with UFH (Positive population, N=50) after treatment with PF4-R and heparinase.

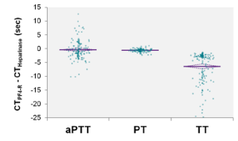

Figure 5. Difference in clot times (mean with 95% confidence interval is indicated) between treatments for each patient sample tested in Figure 4 (N=150). The TT of heparinase treated samples was prolonged by a mean of 6 sec relative to PF4-R.

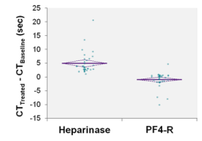

Figure 6. Difference in TT (mean with 95% confidence interval is indicated) between treated and untreated samples (Baseline) from the Target population that did not contain UFH (N=34). Heparinase treated samples showed a mean bias of 5 sec. There was no bias with PF4-R.

References

[1] Harenberg, J. *et al.* Multicentric evaluation of heparinase on aPTT, thrombin clotting time and a new PT reagent based on recombinant human tissue factor. Blood Coagul Fibrinolysis. (1996) 7:453-458.

[2] Cumming, A.M. *et al.* In vitro neutralization of heparin in plasma prior to the activated partial thromboplastin time test: An assessment of four heparin antagonists and two anion exchange resins. Thromb Res. (1986) 41:43-56.

[3] Patent: CA 2810334

[4] Patent pending: US 13/984,335