Unfractionated and LMW Heparin Sensitivity of the CoaguChek® XS PT Test Strip

This paper reviews two heparin sensitivity studies conducted by Roche Diagnostics for the new CoaguChek XS PT Test Strip.

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INTRODUCTION

Roche Diagnostics is introducing the new CoaguChek XS System, which quantitatively determines prothrombin time (PT) in INR, %Quick or Seconds using capillary blood from a fingertip or untreated venous whole blood.

The new CoaguChek XS PT test strip features a lower International Sensitivity Index (ISI) value and chemistry based on human recombinant tissue factor (hrTF).

The test strip chemistry also includes an anti-heparin agent that has been added to neutralize the effects of heparin found in blood samples applied to the test strip.

To evaluate the heparin sensitivity of the CoaguChek XS PT test strip, Roche Diagnostics conducted two tests, one evaluating sensitivity to unfractionated heparin and the other to low molecular weight (LMW) heparin. The study methods, results, and conclusions are presented below.

Test 1: Sensitivity to Unfractionated Heparin

BACKGROUND

Anti-thrombin III inhibits clotting factor proteases by forming equimolar stable complexes with them. In the absence of heparin, these reactions are slow; in the presence of heparin, they are accelerated a thousandfold. Heparin molecules bind tightly to Anti-thrombin III and cause a conformational change in this inhibitor. This conformational change of Anti-thrombin III exposes its active site for rapid interaction with the proteases. This mechanism allows for the anticoagulant properties of heparin.

An anti-heparin agent was added to the chemistry of the CoaguChek XS PT test strip to neutralize the effects of heparin found in the sample applied to the test strip.

In terms of acceptance criteria, the heparin sensitivity for normal donors and warfarin patients is defined as the mean INR at a particular heparin spike level elevated $\leq$ 10% compared to the unspiked sample.

METHOD

The evaluation study was conducted to perform an analysis of the CoaguChek XS PT test strip's sensitivity to unfractionated heparin.[1]

Blood samples from two normal donors and two warfarin patients were spiked with six different heparin levels (0.4, 0.6, 0.8, 1.0, 1.5, 2.0 units). All spiked blood samples were run on the CoaguChek XS System and compared to unspiked blood samples, which were also run on the CoaguChek XS System.

RESULTS/CONCLUSION

All tested concentrations (up to 2 U/mL) from the normal donors showed no interference to unfractionated heparin based on the acceptance criteria of ≤ 10% elevation in mean INR value compared to the unspiked sample.

Blood samples from warfarin patients showed no interference to unfractionated heparin up to 0.8 U/mL based on the acceptance criteria of ≤ 10% INR elevation compared to the unspiked sample.

Normal Donor 01 (Baseline INR 0.95)

Heparin Concentration (units)	Mean Value INR	p Value	Relative Bias [%]
0	0.94		
0.35	0.94	0.678	-0.24
0	0.94		
0.53	0.94	0.811	-0.13
0	0.94		
0.71	0.94	0.075	0.64
0	0.95		
0.89	0.94	0.000	-1.37
0	0.95		
1.33	0.95	0.199	0.48
0	0.95		
1.77	0.97	0.000	1.93

Normal Donor 02 (Baseline INR 1.02)

Heparin Concentration (units)	Mean Value INR	p Value	Relative Bias [%]
0	1.04		
0.39	1.03	0.007	-1.25
0	1.04		
0.59	1.02	0.003	-1.40
0	1.04		
0.78	1.03	0.366	-0.42
0	1.04		
0.98	1.03	0.419	-0.44
0	1.04		
1.46	1.03	0.053	-0.84
0	1.02		
1.95	1.06	0.000	3.70

Warfarin Patient 01 (Baseline INR 2.89)

Heparin Concentration (units)	Mean Value INR	p Value	Relative Bias [%]
0	2.82		
0.42	2.83	0.549	0.41
0	2.89		
0.64	2.89	0.832	-0.17
0	2.87		
0.84	2.91	0.072	1.15
0	2.97		
1.05	3.06	0.000	3.22
0	2.95		
1.57	3.35	0.000	**13.83**
0	2.94		
2.09	4.02	0.000	**36.98**

Warfarin Patient 02 (Baseline INR 2.50)

Heparin Concentration (units)	Mean Value INR	p Value	Relative Bias [%]
0	2.51		
0.40	2.50	0.408	-0.34
0	2.53		
0.61	2.48	0.002	-2.16
0	2.58		
0.81	2.54	0.016	-1.55
0	2.58		
1.02	2.61	0.076	1.20
0	2.52		
1.52	2.82	0.000	**11.86**
0	2.58		
2.02	3.20	0.000	**24.29**

Test 2: Sensitivity to LMW Heparin

BACKGROUND

Low molecular weight heparin is derived from standard heparin through either chemical or enzymatic depolymerization. While standard heparin has a molecular weight of 5,000 to 30,000 daltons, LMW heparin's weight ranges from 1,000 to 10,000 daltons, resulting in properties that are distinct from those of traditional heparin. Low molecular weight heparin binds less strongly to protein, has enhanced bioavailability, interacts less with platelets and yields a very predictable dose response. Low molecular weight heparin, like standard heparin, binds to Anti-thrombin III; however, low molecular weight heparin inhibits thrombin to a lesser degree (and Factor Xa to a greater degree) than standard heparin.

An anti-heparin agent was added to the chemistry of the CoaguChek XS PT test strip to neutralize the effects of heparin found in the sample applied to the test strip.

In terms of acceptance criteria, the heparin sensitivity for normal donors and warfarin patients is defined as the mean INR at a particular heparin spike level elevated $\leq$ 10% compared to the unspiked sample.

METHOD

The evaluation study was conducted to perform an analysis of the CoaguChek XS PT test strip's sensitivity to low molecular weight heparin.[1]

Two different low molecular weight heparin products (Tinzaparin and Enoxaparin) were used – one with a high anti-Xa/anti-IIa ratio and one with a low anti-Xa/anti-IIA ratio.

Blood samples from four normal volunteers (two for Tinzaparin and two for Enoxaparin) and four warfarin patients (two for Tinzaparin and two for Enoxaparin) were spiked with five different heparin levels and compared to an unspiked sample.

RESULTS/CONCLUSION

Blood samples from normal donors and warfarin patients for both Tinzaparin and Enoxaparin heparin showed no interference up to 2 IU anti-factor Xa activity/ml based on the acceptance criteria of $\leq$ 10% INR elevation compared to the unspiked sample.

LMWH: Tinzaparin

Normal Donor T-01 (Baseline INR 0.95)

Heparin Concentration (units)	Mean Value INR	p Value	Relative Bias [%]
0	1.06		
0.59	1.04	0.000	-2.32
0	1.07		
1.18	1.05	0.000	-1.53
0	1.07		
1.75	1.05	0.000	-2.57
0	1.07		
2.31	1.05	0.000	-2.01
0	1.07		
2.93	1.07	0.863	0.07

Normal Donor T-02 (Baseline INR 1.02)

Heparin Concentration (units)	Mean Value INR	p Value	Relative Bias [%]
0	0.95		
0.59	0.95	0.369	0.31
0	0.97		
1.18	0.95	0.000	-2.42
0	0.97		
1.75	0.95	0.000	-2.17
0	0.98		
2.31	0.96	0.000	-2.13
0	0.98		
2.93	0.96	0.000	-2.55

LMWH: Tinzaparin

Warfarin Patient T-01 (Baseline INR 2.89)

Heparin Concentration (units)	Mean Value INR	p Value	Relative Bias [%]
0	2.04		
0.63	2.01	0.075	-1.25
0	2.04		
1.24	2.06	0.056	1.17
0	2.06		
1.85	2.08	0.102	1.29
0	2.09		
2.44	2.17	0.000	4.12
0	2.12		
3.09	2.27	0.000	7.10

Warfarin Patient T-02 (Baseline INR 2.50)

Heparin Concentration (units)	Mean Value INR	p Value	Relative Bias [%]
0	2.12		
0.59	2.08	0.025	-1.85
0	2.10		
1.18	2.11	0.643	0.46
0	2.11		
1.75	2.13	0.106	1.29
0	2.11		
2.31	2.17	0.005	2.79
0	2.12		
2.93	2.29	0.000	7.83

LMWH: Enoxaparin

Normal Donor E-01 (Baseline INR 1.03)

Heparin Concentration (units)	Mean Value INR	p Value	Relative Bias [%]
0	1.02		
0.57	1.02	0.126	-0.82
0	1.02		
1.12	1.01	0.003	-1.61
0	1.03		
1.67	1.01	0.004	-1.75
0	1.02		
2.21	1.03	0.021	1.46
0	1.05		
2.80	1.04	0.303	-0.67

Normal Donor E-02 (Baseline INR 1.02)

Heparin Concentration (units)	Mean Value INR	p Value	Relative Bias [%]
0	1.02		
0.56	1.01	0.109	-1.00
0	1.02		
1.10	1.00	0.000	-2.01
0	1.03		
1.64	1.01	0.000	-2.27
0	1.04		
2.17	1.01	0.000	-2.30
0	1.03		
2.75	1.01	0.014	-1.52

LMWH: Enoxaparin

Warfarin Patient E-01 (Baseline INR 2.08)

Heparin Concentration (units)	Mean Value INR	p Value	Relative Bias [%]
0	2.07		
0.51	2.06	0.122	-0.76
0	2.08		
1.01	2.08	0.987	-0.01
0	2.11		
1.51	2.13	0.176	0.71
0	2.11		
2.00	2.14	0.005	1.47
0	2.10		
2.53	2.18	0.000	3.99

Warfarin Patient E-02 (Baseline INR 2.22)

Heparin Concentration (units)	Mean Value INR	p Value	Relative Bias [%]
0	2.23		
0.57	2.20	0.025	-1.17
0	2.27		
1.12	2.26	0.292	-0.50
0	2.27		
1.67	2.23	0.012	-1.76
0	2.29		
2.21	2.31	0.066	1.02
0	2.28		
2.80	2.33	0.001	2.18

References

1. 510(k) FDA submission for CoaguChek XS system, approved August 2006.

Roche Diagnostics