

Article

# Laboratory Evaluation of Interferences Associated with Factor XIa Inhibitors Asundexian and Milvexian in Routine Coagulation Assays

## Supplementary Material

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**Table S1.** Absolute mean values (standard deviation) of routine coagulation assay parameters in human plasma at select concentrations of asundexian

Assay	Parameter	Reference Range	Asundexian				
			0 ng/mL	250 ng/mL	500 ng/mL	750 ng/mL	1000 ng/mL
<b>Dade® Innovin®</b>	PT (s)	9.5 – 11.1	10.6 (0.1)	10.7 (0.2)	10.7 (0.3)	10.6 (0.2)	10.6 (0.2)
	INR	0.86 – 1.12	0.98 (0.01)	0.99 (0.02)	0.99 (0.03)	0.98 (0.02)	0.98 (0.02)
<b>Thromborel® S</b>	PT (s)	9.6 – 13.0	11.9 (0.3)	11.6 (0.6)	11.6 (0.4)	11.9 (0.5)	11.9 (0.5)
	INR	0.88 – 1.21	1.11 (0.03)	1.08 (0.01)	1.08 (0.02)	1.11 (0.02)	1.11 (0.05)
<b>Dade® Actin® FS</b>	APTT (s)	21.0 – 29.0	26.3 (0.8)	<b>44.6 (3.0)</b>	<b>55.8 (1.4)</b>	<b>62.3 (2.7)</b>	<b>69.1 (3.5)</b>
	APTT-R	0.84 – 1.16	1.05 (0.03)	<b>1.79 (0.12)</b>	<b>2.23 (0.05)</b>	<b>2.49 (0.11)</b>	<b>2.77 (0.14)</b>
<b>Dade® Actin® FSL</b>	APTT (s)	25.0 – 32.0	27.0 (0.5)	<b>39.3 (1.6)</b>	<b>46.3 (2.5)</b>	<b>51.1 (1.0)</b>	<b>56.1 (2.0)</b>
	APTT-R	1.00 – 1.28	1.08 (0.02)	<b>1.57 (0.07)</b>	<b>1.85 (0.12)</b>	<b>2.04 (0.10)</b>	<b>2.24 (0.10)</b>
<b>Dade® Thrombin</b>	FIB (g/L)	1.70 – 4.10	2.58 (0.11)	2.60 (0.09)	2.57 (0.12)	2.62 (0.12)	2.51 (0.10)
<b>INNOVANCE® D-dimer</b>	DDi (mg/L)	< 0.5	0.31 (0.04)	0.28 (0.05)	0.30 (0.04)	0.25 (0.05)	0.31 (0.04)

Data is presented as mean ± standard deviation. Data in bold indicate statistically significant differences (one-way ANOVA with post hoc Dunnett testing:  $p < 0.001$ ) compared to samples without asundexian (0 ng/mL). Abbreviations: APTT, activated partial thromboplastin time; APTT-R, activated partial thromboplastin time ratio; DDi, D-dimer; FIB, fibrinogen; INR, international normalised ratio; PT, prothrombin time. For each assay, five independent replicates of reconstituted standard human-derived plasma were spiked with asundexian or milvexian, respectively, were assayed ( $n = 5$ ).

**Table S2.** Absolute mean values (standard deviation) of routine coagulation assay parameters in human plasma at select concentrations of milvexian

Assay	Parameter	Reference Range	Milvexian				
			0 ng/mL	250 ng/mL	500 ng/mL	750 ng/mL	1000 ng/mL
Dade® Innovin®	PT (s)	9.5 – 11.1	10.7 (0.1)	10.6 (0.2)	10.6 (0.2)	10.7 (0.2)	10.7 (0.2)
	INR	0.96 – 1.12	0.99 (0.02)	0.98 (0.01)	0.98 (0.02)	0.99 (0.02)	0.99 (0.02)
Thromborel® S	PT (s)	9.6 – 13.0	12.1 (0.4)	11.1 (0.5)	11.8 (0.5)	11.6 (0.3)	11.7 (0.2)
	INR	0.88 – 1.21	1.11 (0.05)	1.09 (0.06)	1.1 (0.04)	1.06 (0.03)	1.08 (0.02)
Dade® Actin® FS	APTT (s)	21.0 – 29.0	26.4 (0.7)	<b>47.9 (2.5)</b>	<b>58.1 (1.4)</b>	<b>65 (2.2)</b>	<b>70.8 (2.6)</b>
	APTT-R	0.84 – 1.16	1.05 (0.03)	<b>1.91 (0.10)</b>	<b>2.32 (0.06)</b>	<b>2.6 (0.09)</b>	<b>2.83 (0.11)</b>
Dade® Actin® FSL	APTT (s)	25.0 – 32.0	27.1 (0.2)	<b>41.3 (1.3)</b>	<b>49.5 (3.3)</b>	<b>54.7 (3.4)</b>	<b>58.1 (2.8)</b>
	APTT-R	1.00 – 1.28	1.08 (0.01)	<b>1.65 (0.06)</b>	<b>1.98 (0.13)</b>	<b>2.19 (0.15)</b>	<b>2.32 (0.13)</b>
Dade® Thrombin	FIB (g/L)	1.70 – 4.10	2.54 (0.11)	2.54 (0.13)	2.64 (0.12)	2.61 (0.09)	2.54 (0.13)
INNOVANCE® D-dimer	DDi (mg/L)	< 0.5	0.26 (0.07)	0.29 (0.07)	0.29 (0.06)	0.27 (0.01)	0.24 (0.03)

Data is presented as mean ± standard deviation. Data in bold indicate statistically significant differences (one-way ANOVA with post hoc Dunnett testing;  $p < 0.0001$ ) compared to samples without asundexian (0 ng/mL). Abbreviations: APTT, activated partial thromboplastin time; APTT-R, activated partial thromboplastin time ratio; DDi, D-dimer; FIB, fibrinogen; INR, international normalised ratio; PT, prothrombin time. For each assay, five independent replicates of reconstituted standard human-derived plasma were spiked with asundexian or milvexian, respectively, were assayed ( $n = 5$ ).

**Table S3.** Absolute mean values (standard deviation) of routine coagulation assay parameters in Control and Calibrator Material at select concentrations of asundexian

Assay	Control and Calibrator	Parameter	Reference Range	Asundexian				
				0 ng/mL	250 ng/mL	500 ng/mL	750 ng/mL	1000 ng/mL
Dade® Innovin®	Ci-trol® 2	PT (s)	9.5 – 11.1	26.3 (0.5)	26.2 (0.3)	28.0 (0.9)	26.8 (0.6)	26.1 (0.5)
		INR	0.86 – 1.12	2.63 (0.05)	2.63 (0.03)	2.83 (0.09)	2.69 (0.07)	2.61 (0.05)
Dade® Innovin®	Ci-trol® 3	PT (s)	9.5 – 11.1	46.8 (1.1)	45.9 (1.0)	47.8 (1.0)	46.6 (1.1)	46.7 (1.2)
		INR	0.86 – 1.12	4.94 (0.1)	4.84 (0.1)	5.06 (0.1)	4.92 (0.1)	4.94 (0.1)
Thromborel® S	Ci-trol® 2	PT (s)	9.6 – 13.0	28.5 (0.4)	28.9 (0.2)	27.6 (0.9)	27.1 (0.5)	28.9 (0.6)
		INR	0.88 – 1.21	2.82 (0.04)	2.90 (0.02)	2.80 (0.10)	2.85 (0.06)	2.82 (0.07)
Thromborel® S	Ci-trol® 3	PT (s)	9.6 – 13.0	47.2 (0.2)	48.0 (0.8)	48.0 (0.8)	48.6 (0.3)	47.5 (0.7)
		INR	0.88 – 1.21	4.99 (0.02)	5.08 (0.09)	5.08 (0.10)	5.16 (0.03)	5.02 (0.08)
Dade® Actin® FS	Ci-trol® 2	APTT (s)	21.0 – 29.0	45.9 (0.7)	<b>51.8 (0.9)</b>	<b>70.0 (1.6)</b>	<b>86.0 (3.0)</b>	<b>91.6 (1.7)</b>
		APTT-R	0.84 – 1.16	1.83 (0.03)	<b>2.07 (0.03)</b>	<b>2.80 (0.06)</b>	<b>3.35 (0.12)</b>	<b>3.56 (0.06)</b>
Dade® Actin® FS	Ci-trol® 3	APTT (s)	21.0 – 29.0	63.7 (0.5)	<b>76.2 (3.5)</b>	<b>121. (6.7)</b>	<b>146.1 (5.3)</b>	<b>165.2 (2.3)</b>
		APTT-R	0.84 – 1.16	2.54 (0.02)	<b>3.04 (0.14)</b>	<b>4.85 (0.26)</b>	<b>5.85 (0.21)</b>	<b>6.60 (0.09)</b>
Dade® Actin® FSL	Ci-trol® 2	APTT (s)	25.0 – 32.0	44.4 (1.1)	<b>51.9 (0.5)</b>	<b>59.5 (0.3)</b>	<b>58.6 (6.5)</b>	<b>74.6 (3.6)</b>
		APTT-R	1.00 – 1.28	1.77 (0.04)	<b>2.07 (0.02)</b>	<b>2.38 (0.01)</b>	<b>2.34 (0.26)</b>	<b>2.98 (0.14)</b>
Dade® Actin® FSL	Ci-trol® 3	APTT (s)	25.0 – 32.0	68.1 (1.5)	<b>72.0 (1.9)</b>	<b>83.2 (1.1)</b>	<b>101.0 (3.5)</b>	<b>133.5 (4.4)</b>
		APTT-R	1.00 – 1.28	2.72 (0.06)	<b>2.88 (0.07)</b>	<b>3.32 (0.04)</b>	<b>4.04 (0.14)</b>	<b>5.34 (0.17)</b>
Dade® Thrombin	Control Plasma P	FIB (g/L)	1.70 – 4.10	0.73 (0.04)	0.71 (0.02)	0.72 (0.03)	0.66 (0.02)	0.71 (0.04)
INNOVANCE® D-dimer	Control 2	DDi (mg/L)	< 0.5	2.49 (0.04)	2.62 (0.16)	2.57 (0.15)	2.5 (0.10)	2.44 (0.05)

Data is presented as mean ± standard deviation. Data in bold indicate statistically significant differences (one-way ANOVA with post hoc Dunnett testing:  $p < 0.001$ ) compared to samples without asundexian (0 ng/mL). Abbreviations: APTT, activated partial thromboplastin time; APTT-R, activated partial thromboplastin time ratio; DDi, D-dimer; FIB, fibrinogen; INR, international normalised ratio; PT, prothrombin time. For each assay, three independent replicates of reconstituted standard human-derived plasma were spiked with asundexian or milvexian, respectively, were assayed ( $n = 3$ ).

**Table S4.** Absolute mean values (standard deviation) of routine coagulation assay parameters in Control and Calibrator Material at select concentrations of milvexian

			Milvexian					
Assay		Parameter	Reference Range	0 ng/mL	250 ng/mL	500 ng/mL	750 ng/mL	1000 ng/mL
Dade® Innovin®	Ci-trol® 2	PT (s)	9.5 – 11.1	26.4 (1.4)	26.6 (1.3)	26.2 (1.2)	25.9 (0.3)	26.4 (1.1)
		INR	0.86 – 1.12	2.64 (0.15)	2.67 (0.15)	2.63 (0.13)	2.60 (0.03)	2.64 (0.12)
Dade® Innovin®	Ci-trol® 3	PT (s)	9.5 – 11.1	45.9 (1.2)	45.6 (1.7)	46.4 (1.8)	46.6 (1.0)	44.9 (0.7)
		INR	0.86 – 1.12	4.84 (0.14)	4.81 (0.20)	4.90 (0.21)	4.92 (0.12)	4.73 (0.08)
Thromborel® S	Ci-trol® 2	PT (s)	9.6 – 13.0	27.1 (0.4)	28.8 (0.6)	28.7 (0.3)	28 (0.6)	28.2 (0.6)
		INR	0.88 – 1.21	2.85 (0.05)	2.80 (0.05)	2.83 (0.02)	2.86 (0.02)	2.85 (0.07)
Thromborel® S	Ci-trol® 3	PT (s)	9.6 – 13.0	47.2 (0.2)	48.0 (0.8)	48.3 (0.9)	48.6 (0.3)	47.5 (0.7)
		INR	0.88 – 1.21	4.99 (0.02)	5.08 (0.09)	5.12 (0.10)	5.16 (0.03)	5.02 (0.08)
Dade® Actin® FS	Ci-trol® 2	APTT (s)	21.0 – 29.0	43.0 (0.5)	<b>51.8 (1.0)</b>	<b>69.0 (2.0)</b>	<b>89.0 (1.3)</b>	<b>104.4 (1.3)</b>
		APTT-R	0.84 – 1.16	1.72 (0.02)	<b>2.07 (0.04)</b>	<b>2.76 (0.08)</b>	<b>3.56 (0.05)</b>	<b>4.17 (0.05)</b>
Dade® Actin® FS	Ci-trol® 3	APTT (s)	21.0 – 29.0	68.2 (1.4)	<b>105.3 (9.0)</b>	<b>140.3 (7.6)</b>	<b>163.5 (2.6)</b>	<b>187.4 (1.3)</b>
		APTT-R	0.84 – 1.16	2.72 (0.05)	<b>3.96 (0.36)</b>	<b>5.61 (0.30)</b>	<b>6.54 (0.10)</b>	<b>7.49 (0.05)</b>
Dade® Actin® FSL	Ci-trol® 2	APTT (s)	25.0 – 32.0	43.4 (1.2)	<b>51.2 (0.4)</b>	<b>62.9 (0.4)</b>	<b>77.9 (2.2)</b>	<b>85.8 (1.1)</b>
		APTT-R	1.00 – 1.28	1.73 (0.05)	<b>2.04 (0.01)</b>	<b>2.51 (0.01)</b>	<b>3.11 (0.09)</b>	<b>3.43 (0.04)</b>
Dade® Actin® FSL	Ci-trol® 3	APTT (s)	25.0 – 32.0	65.8 (1.7)	<b>78.0 (3.5)</b>	<b>92.7 (0.7)</b>	<b>108.0 (1.8)</b>	<b>137.8 (7.4)</b>
		APTT-R	1.00 – 1.28	2.63 (0.06)	<b>3.12 (0.14)</b>	<b>3.71 (0.02)</b>	<b>4.32 (0.07)</b>	<b>5.51 (0.29)</b>
Dade® Thrombin	Control Plasma P	FIB (g/L)	1.70 – 4.10	0.73 (0.04)	0.71 (0.02)	0.72 (0.03)	0.66 (0.02)	0.71 (0.04)
INNOVANCE® D-dimer	Control 2	DDi (mg/L)	< 0.5	2.49 (0.04)	2.62 (0.16)	2.57 (0.15)	2.50 (0.09)	2.44 (0.05)

Data is presented as mean ± standard deviation. Data in bold indicate statistically significant differences (one-way ANOVA with post hoc Dunnett testing:  $p < 0.001$ ) compared to samples without asundexian (0 ng/mL). Abbreviations: APTT, activated partial thromboplastin time; APTT-R, activated partial thromboplastin time ratio; DDi, D-dimer; FIB, fibrinogen; INR, international normalised ratio; PT, prothrombin time. For each assay, three independent replicates of reconstituted standard human-derived plasma were spiked with asundexian or milvexian, respectively, were assayed ( $n = 3$ ).

**Table S5.** Absolute mean values (standard deviation) of APTT-based assay parameters in human plasmas at different concentrations of asundexian

Assay	Parameter	Reference Range	Asundexian				
			0 ng/mL	1.565 ng/mL	3.125 ng/mL	6.25 ng/mL	12.5 ng/mL
Dade® Actin® FS	APTT (s)	21.0 – 29.0	27.0 (3.6)	27.2 (3.5)	27.5 (3.4)	28.3 (3.2)	29.2 (3.0)
	APTT-R	0.96 – 1.12	1.08 (0.15)	1.09 (0.14)	1.10 (0.14)	1.13 (0.13)	1.16 (0.12)
Dade® Actin® FS	FXI (%)	67 - 127	87.5 (2.7)	87.2 (2.7)	86.9 (2.5)	85.9 (3.0)	85.5 (2.6)
Assay	Parameter	Reference Range	25 ng/mL	50 ng/mL	100 ng/mL	200 ng/mL	300 ng/mL
Dade® Actin® FS	APTT (s)	21.0 – 29.0	31.0 (2.8)	34.0 (2.5)	39.4 (3.4)	47.8 (3.0)	50.3 (4.5)
	APTT-R	0.96 – 1.12	1.24 (0.11)	1.36 (0.10)	1.57 (0.14)	1.91 (0.12)	2.01 (0.18)
Dade® Actin® FS	FXI (%)	67 - 127	83.1 (3.0)	79.4 (3.2)	72.1 (3.1)	61.8 (3.9)	62.8 (12.)
Assay	Parameter	Reference Range	400 ng/mL	500 ng/mL	600 ng/mL	700 ng/mL	800 ng/mL
Dade® Actin® FS	APTT (s)	21.0 – 29.0	58.5 (3.9)	62.4 (4.2)	65.8 (4.0)	68.4 (4.4)	71.0 (4.1)
	APTT-R	0.96 – 1.12	2.34 (0.16)	2.49 (0.17)	2.63 (0.16)	2.73 (0.18)	2.84 (0.17)
Dade® Actin® FS	FXI (%)	67 - 127	47.6 (5.5)	43.0 (6.5)	38.6 (7.4)	35.3 (7.9)	32.9 (7.7)
Assay	Parameter	Reference Range	900 ng/mL	1000 ng/mL	2000 ng/mL	3000 ng/mL	4000 ng/mL
Dade® Actin® FS	APTT (s)	21.0 – 29.0	72.8 (4.6)	75.1 (4.4)	84.0 (5.3)	91.6 (6.3)	96.8 (5.6)
	APTT-R	0.96 – 1.12	2.91 (0.19)	3.00 (0.18)	3.36 (0.21)	3.66 (0.25)	3.87 (0.23)
Dade® Actin® FS	FXI (%)	67 - 127	30.7 (7.7)	29.0 (7.4)	22.9 (6.0)	16.4 (3.4)	14.1 (3.4)

Data is presented as mean ± standard deviation. Abbreviations: APTT, activated partial thromboplastin time; APTT-R, activated partial thromboplastin time ratio; FXI, factor XI. For each assay, five independent replicates of five different reconstituted human-derived plasmas (Standard Human Plasma, Ci-trol® 1, Control Plasma N, BIOPHEN™ Plasma Calibrator, and BIOPHEN™ Normal Control Plasma) were spiked with asundexian or milvexian, respectively, were assayed (N = 5; n = 5).

**Table S6.** Absolute mean values (standard deviation) of APTT-based assay parameters in human plasmas at different concentrations of milvexian

Assay	Parameter	Reference Range	Milvexian				
			0 ng/mL	1.565 ng/mL	3.125 ng/mL	6.25 ng/mL	12.5 ng/mL
Dade® Actin® FS	APTT (s)	21.0 – 29.0	26.9 (3.1)	27.5 (3.0)	27.9 (3.0)	28.7 (3.0)	29.7 (2.9)
	APTT-R	0.96 – 1.12	1.07 (0.13)	1.10 (0.12)	1.11 (0.12)	1.15 (0.21)	1.18 (0.12)
Dade® Actin® FS	FXI (%)	67 - 127	87.2 (6.3)	87.2 (6.1)	86.8 (6.0)	85.9 (6.0)	85.1 (5.8)
Assay	Parameter	Reference Range	25 ng/mL	50 ng/mL	100 ng/mL	200 ng/mL	300 ng/mL
Dade® Actin® FS	APTT (s)	21.0 – 29.0	31.8 (2.8)	34.8 (2.9)	40.1 (3.1)	49.8 (2.9)	46.4 (6.4)
	APTT-R	0.96 – 1.12	1.27 (0.12)	1.39 (0.12)	1.60 (0.13)	1.99 (0.12)	1.85 (0.26)
Dade® Actin® FS	FXI (%)	67 - 127	82.6 (5.5)	78.4 (5.2)	70.1 (5.3)	59.1 (5.9)	55.7 (12.)
Assay	Parameter	Reference Range	400 ng/mL	500 ng/mL	600 ng/mL	700 ng/mL	800 ng/mL
Dade® Actin® FS	APTT (s)	21.0 – 29.0	57.4 (4.2)	61.3 (4.0)	65.7 (3.5)	68.8 (3.3)	71.9 (3.3)
	APTT-R	0.96 – 1.12	2.29 (0.17)	2.45 (0.16)	2.63 (0.14)	2.75 (0.13)	2.87 (0.14)
Dade® Actin® FS	FXI (%)	67 - 127	44.5 (7.1)	39.8 (7.5)	35.7 (7.8)	32.7 (8.0)	30.4 (7.9)
Assay	Parameter	Reference Range	900 ng/mL	1000 ng/mL	2000 ng/mL	3000 ng/mL	4000 ng/mL
Dade® Actin® FS	APTT (s)	21.0 – 29.0	74.3 (3.5)	77.7 (3.5)	87.7 (4.3)	100. (6.5)	108. (8.2)
	APTT-R	0.96 – 1.12	2.97 (0.14)	3.11 (0.14)	3.50 (0.17)	4.02 (0.26)	4.35 (0.33)
Dade® Actin® FS	FXI (%)	67 - 127	28.4 (7.8)	26.7 (7.8)	20.7 (6.5)	15.6 (4.1)	13.7 (3.8)

Data is presented as mean ± standard deviation. Abbreviations: APTT, activated partial thromboplastin time; APTT-R, activated partial thromboplastin time ratio; FXI, factor XI. For each assay, five independent replicates of five different reconstituted human-derived plasmas (Standard Human Plasma, Ci-trol® 1, Control Plasma N, BIOPHEN™ Plasma Calibrator, and BIOPHEN™ Normal Control Plasma) were spiked with asundexian or milvexian, respectively, were assayed (N = 5; n = 5).

**Table S7.** Multi-dilutional analysis of factor XI activity in human plasmas at 1000 ng/mL of asundexian and milvexian

Assay	Parameter	Reference Range	Asundexian (1000 ng/mL)			Milvexian (1000 ng/mL)		
			Dilutions			Dilutions		
			1:1	1:2	1:4	1:1	1:2	1:4
<b>Dade® Actin® FS</b>	FXI (%)	67 - 127	29.0 (7.4)	35.2 (6.1)	44.9 (6.5)	26.7 (7.8)	34.2 (4.1)	41.9 (4.5)

Data is presented as mean  $\pm$  standard deviation. Dilutions are presented as the volumetric ratio of asundexian- or milvexian-containing plasma and factor XI-deficient plasma assayed. Abbreviations: FXI, factor XI. For each assay, five independent replicates of five different reconstituted human-derived plasmas (Standard Human Plasma, Ci-trol® 1, Control Plasma N, BIOPHEN™ Plasma Calibrator, and BIOPHEN™ Normal Control Plasma) were spiked with asundexian or milvexian, respectively, were assayed (N = 5; n = 5).