

Evaluation Report
MRX147 D-Dimer on Coatron M4
Performance Characteristics

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Introduction

The performance data below was collected on a Coatron M4 (TECO Application sheet for D-DIMER MRX147 analysis Rev. 18.08.2008). The results are transferrable to Coatron M2, Techrome IV (405 nm filter), Humaclot Duo and Humaclot Jr .

Reagent Information

MRX147 D-dimer is a micro-particle enhanced immunoassay for the quantitative determination of the fibrin degradation product D-dimer in human plasma. MRX147 D-dimer consists of a pair of components, Latex Reagent and Reaction Buffer. When these two reagents are mixed, together with a D-dimer containing plasma sample, the particles will agglutinate and give rise to an increase in light scattering. When this reaction is monitored in an instrument, having the necessary photometric features, it will be observed as an increase in absorbance over time. The rate of this reaction is proportional to the amount of D-dimer antigen in the sample.

By virtue of its particles size, MRX147 D-dimer is suitable for instruments with optical channels in the range 400 – 600 nm. At higher wavelengths, the light scattering properties of the particles decrease, which will lower the signal. Conversely, at lower wavelengths the particles would scatter too much light, reducing the residual signal seen by the detector close to its noise level.

MRX147 D-dimer Latex Reagent is manufactured by covalent coupling of D-dimer specific monoclonal antibodies to sub-micron sized particles. The particles are suspended in a buffer containing blockers to reduce non-specific interactions and preservatives to prolong shelf-life.

MRX147 D-dimer Reactions Buffer contains agents that facilitate particle agglutination. For each batch of Latex Reagent, a specific batch of Reaction Buffer is also produced and fine-tuned to render the particles the same specific agglutination rate, lot-to-lot.

Performance – instrument specific

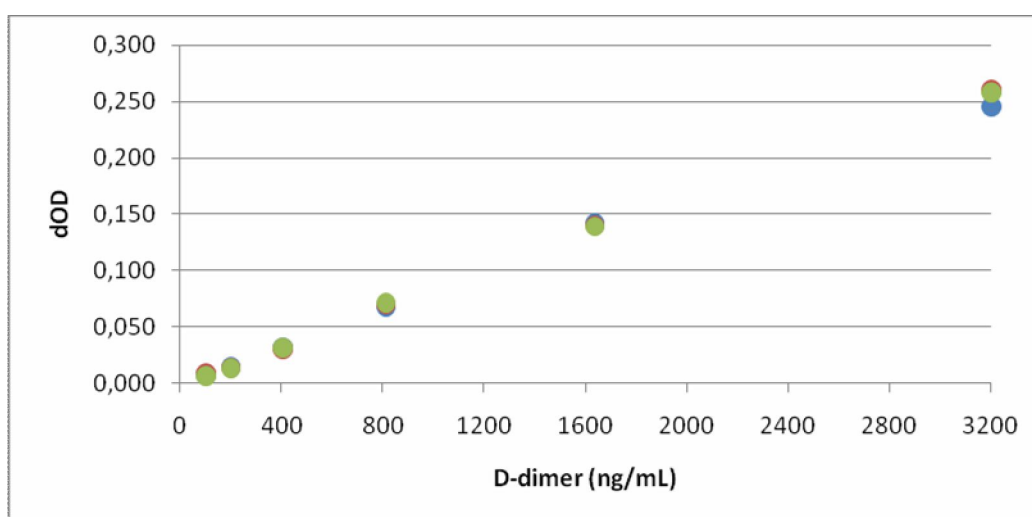
Calibration curve

To demonstrate the system's ability to reproducibly construct calibration curves, three separate calibration curves were established. D-dimer Calibrator 3200 (MRX144) was diluted with Dilution Buffer (lot # 82307), using an analytical balance, to obtain the calibration samples. To construct each calibration curve, the mean of a duplicate run was used. The three curves (Table 1, Figure 1) are well in agreement with each other. The calibration curves are linear over the range. The software of the instrument uses a point-to-point function for the calibration data.

Table 1: Data for three separate calibration curves

DD (ng/mL)	dOD Curve 1	dOD Curve 2	dOD Curve 3	Average	Std. Dev.	CV
99	0.007	0.009	0.006	0.007	0.0013	17.6%
199	0.015	0.014	0.012	0.014	0.0008	5.5%
402	0.032	0.031	0.031	0.031	0.0008	2.5%
810	0.068	0.070	0.071	0.070	0.0015	2.2%
1634	0.142	0.140	0.139	0.140	0.0015	1.1%
3202	0.243	0.260	0.258	0.255	0.0076	3.0%
Slope	7.80×10^{-5}	8.21×10^{-5}	8.17×10^{-5}	8.06×10^{-5}	2.23×10^{-7}	2.8%
Correlation	0.995	0.999	0.998	0.997	-	-

Figure 1: Three superimposed calibration curves



Reportable range

The reportable range of MRX147 D-dimer on Coatron M4 is 100 – 5000 ng/mL. Higher values, reported by the instrument as >5000 (or xxx), should be diluted and re-assayed. There is no prozone effect below 100 000 ng/mL. Please find the data below, supporting these statements.

Lower detection limit

Six samples in the lower range were assayed six times each, using the Sysmex CA-1500. The lower limit of detection (functional sensitivity) was taken as the lowest D-dimer concentration at which the coefficient of variation was better than 20%.

Table 2: Variation of samples in the lower range (ng/mL)

Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Average	Std. Dev.	CV
444	433	454	465	475	475	458	17.1	3.7%
211	235	235	199	271	223	229	24.9	10.9%
115	103	127	115	115	163	123	21.0	17.1%
80	115	115	103	80	68	96	17.5	18.4%
68	68	68	92	68	56	70	11.8	16.9%
44	56	56	56	44	68	54	9.0	16.7%
56	44	0	0	56	0	26	28.8	111%

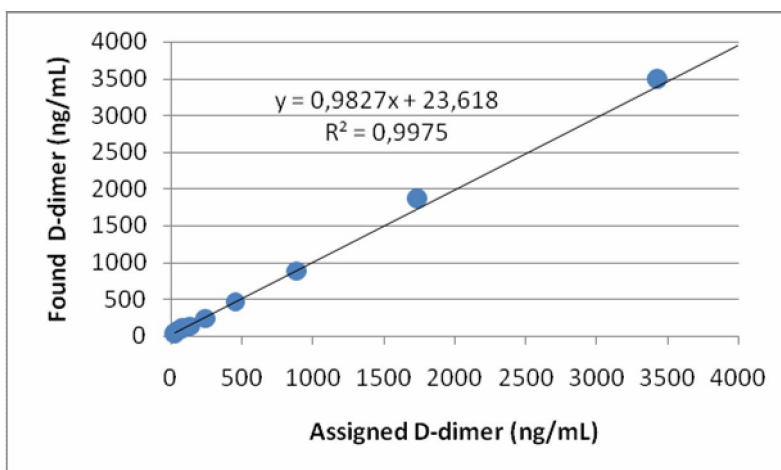
High-dose response

A dilution series of seventeen samples, with a wide range of D-dimer concentrations, was prepared. These samples were assayed on the Coatron M4 (Table 3, Figure 2). Up to 4000 ng/mL, all assays results line up on a Found/Assigned-slope close to unity. For higher values, a feature built into the instrument software, detects the non-linearity within the time-window of the measurement, and reports >5000 (or xxx). This prevents high samples being falsely reported within the reportable range. In other words, there is no prozone effect below 100 000 ng/mL.

Table 3: High-dose response

D-dimer (ng/mL)	
Assigned	Found
23	26
38	54
50	70
77	96
131	123
239	229
454	458
881	881
1735	1871
3425	3500
4270	4079
6804	>5000
13521	>5000
26772	>5000
53455	XXX
106345	XXX

Figure 2: Found D-dimer values versus assigned



Precision

The precision of MRX147 D-dimer on Coatron M4 was determined by running two samples, one in the lower range and another in the middle of the measuring range, at seven different occasions. At each occasion, the samples were assayed six times each. Moreover, these occasions were divided over four different days, with a new calibration each day. The data below (Table 4, Table 5) demonstrate good precision with a total variation of 9.3% in the lower range, and 5.0% in the middle of the measuring range.

Table 4: Precision data in the lower measuring range (ng/mL)

	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7		
	290	303	301	301	210	307	239	runs	n = 7
	264	277	313	277	283	283	264	replicates	k = 6
	303	277	228	301	295	283	277		
	264	264	301	301	271	271	264		
	303	290	313	265	295	295	290		
	277	290	204	289	295	307	239	Mean	
Mean	284	284	277	289	275	291	262	280	SD (of mean)= 9.9
Variance	312	181	2316	238	1118	220	421	686	Var (of mean)= 97.1
SD	17.7	13.4	48.1	15.4	33.4	14.8	20.5	26.2	
CV %	6.2	4.7	17.4	5.3	12.2	5.1	7.8	9.3	
Within run									
Between run									
Total									
Variance	686				16.2			Variance	671
SD	26.2				4.0			SD	25.9
CV %	9.4				1.4			CV %	9.3
dF, n(k-1)	35				6			dF, (n x k)-1	41

Table 5: Precision data in the middle of the measuring range (ng/mL)

	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7		
	1700	1712	1544	1593	1482	1568	1623	runs	n = 7
	1700	1776	1581	1520	1543	1555	1751	replicates	k = 6
	1764	1725	1544	1642	1555	1641	1674		
	1725	1700	1678	1678	1592	1690	1571		
	1700	1725	1617	1630	1568	1519	1623		
	1661	1648	1605	1605	1519	1519	1815	Mean	
Mean	1708	1715	1595	1611	1555	1582	1676	1635	SD (of mean)= 64.1
Variance	1160	1735	2579	2896	748	4815	8303	3176	Var (of mean)= 4115
SD	34.1	41.7	50.8	53.8	27.4	69.4	91.1	56.4	
CV %	2.0	2.4	3.2	3.3	1.8	4.4	5.4	3.4	
Within run									
Between run									
Total									
Variance	3177				3585			Variance	6718
SD	56.4				59.9			SD	82.0
CV %	3.5				3.7			CV %	5.0
dF, n(k-1)	35				6			dF, (n x k)-1	41

Interfering substances

Hemoglobin

A D-dimer control in the middle of the reportable range was divided into six sample tubes. Each tube was spiked with different concentrations of Hemoglobin (Sigma H7379, 048K7555). The assay results (Table 6) show that MRX147 D-dimer is affected by Hemoglobin concentrations above 4 g/L. At the wavelength which the Coatron M4 operates (400 nm), Hemoglobin has its prime adsorption peak. For this reason, the light-transmittance is very low in the highest Hemoglobin concentrations tested, resulting in poor precision. However, for normal samples this is not a problem as the hemoglobin concentration in normal samples is usually in the range 0.02-0.03 g/L. In conclusion, Hemoglobin has no effect on the assay below 4 g/L.

Table 6: Effect of Hemoglobin on a D-dimer control

Hemoglobin g/L	Replicate 1 ng/mL	Replicate 2 ng/mL	Replicate 3 ng/mL	Replicate 4 ng/mL	Mean ng/mL	Recovery
0.0	768	758	747	768	760	100%
2.0	779	768	747	822	779	102%
4.0	800	726	779	779	771	101%
6.0	622	768	674	643	677	89%
8.0	726	465	758	548	624	82%
10.0	390	528	378	622	480	63%

Bilirubin

A D-dimer control in the middle of the reportable range was divided into six sample tubes. Each tube was spiked with different concentrations of Bilirubin (Sigma B4126; 016K1364). The assay results (Table 7) show that MRX147 D-dimer is affected by Bilirubin concentrations above 0.1 g/L. Just like for Hemoglobin, the absorbance of Bilirubin is high at 400 nm resulting in poor light transmittance. The Bilirubin concentration in normal samples is below 0.012 g/L. In conclusion, Bilirubin has no effect on the assay below 0.1 g/L.

Table 7: Effect of Bilirubin on a D-dimer control

Bilirubin g/L	Replicate 1 ng/mL	Replicate 2 ng/mL	Replicate 3 ng/mL	Replicate 4 ng/mL	Mean ng/mL	Recovery
0.0	845	726	800	834	801	100%
0.1	737	653	768	758	729	91%
0.2	664	590	559	569	596	74%
0.3	402	390	611	695	525	65%

Lipids

A D-dimer control in the middle of the reportable range was divided into ten sample tubes. Each tube was spiked with different concentrations of Intralipid (Sigma I141; 117K07251). The assay results (Table 8) show that MRX147 D-dimer is affected by lipids; by linear interpolation the recovery is 90% at 2.5 g/L. The triglyceride concentration in normal samples is below 2.5 g/L, in hypertriglyceridemic samples above 5.0 g/L, and in samples from patients with high risk for pancreatitis above 10 g/L. Hence, with MRX147 D-dimer, high lipemic samples should be diluted and re-assayed.

Table 8: Effect of Lipids on a D-dimer control

Triglycerides g/L	Replicate 1 ng/mL	Replicate 2 ng/mL	Replicate 3 ng/mL	Replicate 4 ng/mL	Mean ng/mL	Recovery
1.0	758	779	768	747	763	100%
2.9	643	643	664	674	656	86%
4.0	548	664	611	590	603	79%
7.0	507	465	486	454	478	63%
9.4	259	330	306	306	300	39%
12.0	223	235	259	259	244	32%

Heparin (non-fractionated)

A D-dimer control in the middle of the reportable range was divided into four sample tubes. Each tube was spiked with different concentrations of non-fractionated Heparin (Heparin LEO, DC6360). The assay results (Table 9) show that MRX147 D-dimer is only modestly affected by any of the tested Heparin concentrations.

Table 9: effect of non-fractionated Heparin on a D-dimer control

Heparin U/mL	Replicate 1 ng/mL	Replicate 2 ng/mL	Replicate 3 ng/mL	Replicate 4 ng/mL	Mean ng/mL	Recovery
0	800	810	768	810	797	100%
1	747	810	810	822	797	100%
10	753	857	845	869	831	104%
100	881	800	800	904	846	106%

Heparin (low molecular weight)

A D-dimer control in the middle of the reportable range was divided into four sample tubes. Each tube was spiked with different concentrations of LMW Heparin (Fragmin, Pfizer 8Q0007). The assay results (Table 10) show that MRX147 D-dimer is unaffected by any of the tested Heparin concentrations.

Table 10: Effect of low-molecular weight Heparin on a D-dimer control

Fragmin U/mL	Replicate 1 ng/mL	Replicate 2 ng/mL	Replicate 3 ng/mL	Replicate 4 ng/mL	Mean ng/mL	Recovery
0	768	758	789	810	781	100%
1	758	768	779	758	766	98%
10	810	716	822	737	771	99%
100	768	800	779	834	795	102%

Correlation

Samples from 66 patients (referred for D-dimer testing to the Umeå University Hospital) were assayed in parallel with two different D-dimer methods: MediRox MRX147 D-dimer (lot # 00108242) on Coatron M4 and GHI143 (Trinity Biotech Auto-Dimer) (lot # S147053) on Sysmex CA-1500. The data (Table 11, Figure 3) show good correlation between the two methods:

$$\text{MRX147 D-dimer} = 1.07 \times \text{Auto-dimer} - 17.5; R^2 = 0.99$$

Figure 3: Correlation MRX147 D-dimer versus GHI143 (AutoDimer)

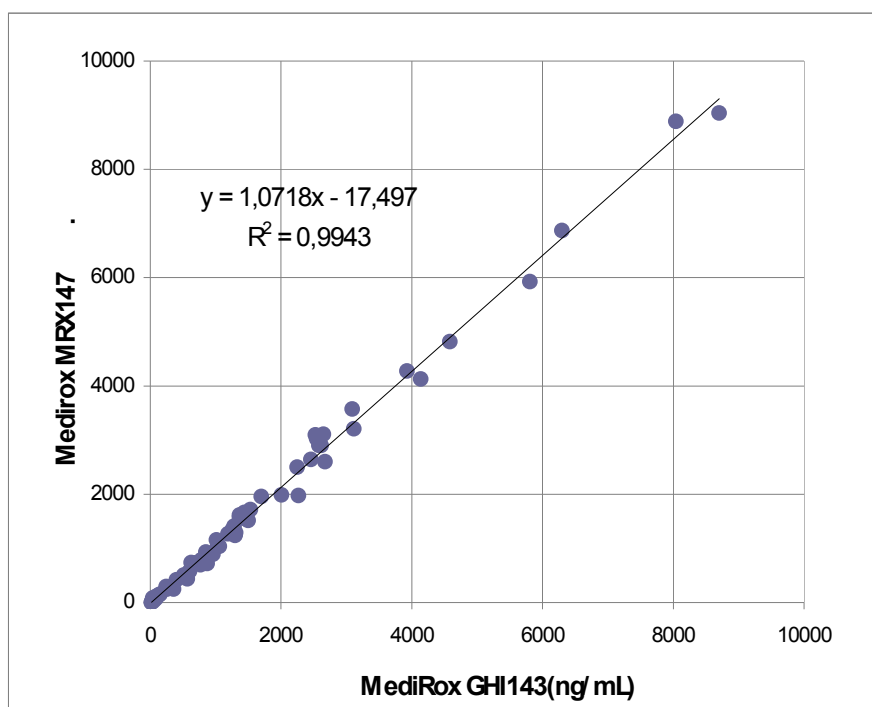


Table 11: Correlation

Trinity Biotech Auto-Dimer	MediRox MRX147 DD
21	0
21	0
36	44
39	80
50	22
54	56
89	68
96	115
136	139
154	151
243	295
250	259
250	271
257	247
279	259
279	283
357	247
403	418
515	507
567	437
600	580
626	737
669	732
770	695
780	786
811	781
830	823
854	934
870	722
959	893
1014	1157
1035	1045
1057	1039
1190	1269
1252	1339
1281	1410
1296	1239
1300	1257
1307	1292
1365	1617
1368	1574
1440	1661
1460	1542
1499	1516
1531	1715
1699	1960
2008	1987
2249	2499
2267	1974
2459	2642
2527	3098
2545	3023
2582	2900
2613	2901
2650	3105
2675	2601
3089	3575
3114	3207
3928	4275
4138	4125
4584	4820
5806	5923
6300	6870
8043	8885
8700	9038

Performance - instrument unrelated

Specificity

For a detailed study of the specificity of the monoclonal antibody in MRX147 D-dimer, please confer separate Technical Report.

Stability

Please confer separate Technical Report for a detailed account of the MRX147 D-dimer shelf-life and in-use stability.

