

Evaluation Report

MRX143 D-Dimer on Sysmex CA-1500/7000

Performance Characteristics

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Introduction

The performance data below was collected on a Sysmex CA-1500. As Sysmex CA-7000 has identical optics and functions, compared to CA-1500, the data is valid also for this instrument.

Reagent Information

MRX143 is a micro-particle enhanced immunoassay for the quantitative determination of the fibrin degradation product D-dimer in human plasma. MRX143 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, MRX143 is suitable for instruments with optical channels in the range 600 – 900 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.

MRX143 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.

MRX143 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 instruments ability to reproducibly construct calibration curves, three separate calibration curves were established using the auto dilution mode. The three curves (Table 1, Figure 1) are well in agreement with each other.

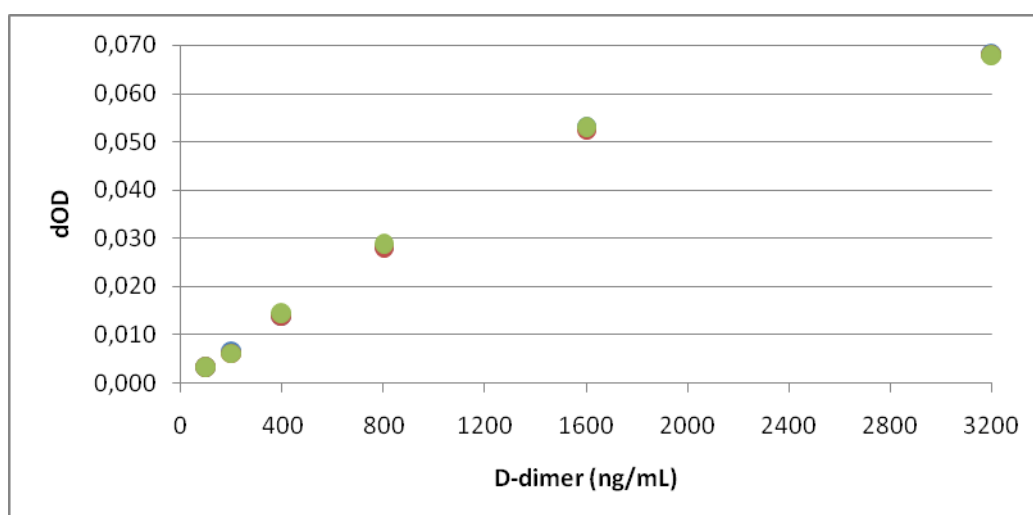
With the current application on Sysmex CA-1500/7000, the slope of the calibration curve has been increased in the lower region (0 – 1600 ng/mL), to increase dose-response where it is most important, at the expense of accuracy in the higher region (> 1600 ng/mL), where the exact assay value is of less importance. A point-to-point calibration curve is established by the instrument.

Table 1: Data for three separate calibration curves

DD (ng/mL)	dOD Curve 1	dOD Curve 2	dOD Curve 3	Average	Std. Dev.	CV
100	0.0031	0.0032	0.0032	0.0032	0.00006	1.8%
200	0.0067	0.0062	0.0062	0.0064	0.00029	4.5%
400	0.0138	0.0139	0.0143	0.0140	0.00026	1.9%
800	0.0283	0.0279	0.0287	0.0283	0.00040	1.4%
1600	0.0532	0.0524	0.0529	0.0528	0.00040	0.8%
3200	0.0680	0.0681	0.0677	0.0679	0.00021	0.3%
Slope*	3.34×10^{-5}	3.30×10^{-5}	3.33×10^{-5}	3.32×10^{-5}	2.52×10^{-7}	0.8%
Correlation**	0.999	0.999	0.998	0.998	-	-

* The slope of the calibration curve in the 0 – 1600 ng/mL range
 ** Correlation coefficient (R^2) or a linear fit to the data in the 0 – 1600 ng/mL range

Figure 1: Three superimposed calibration curves



Reportable range

The reportable range of MRX143 on Sysmex CA-1500/7000 is 50 – 3400 ng/mL. Higher values, reported by the instrument as *Range Over* (or *No Linearity*), should be diluted and re-assayed. There is no prozone effect below 130 000 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
294	289	286	301	304	301	296	7,30	2,5%
180	160	180	177	163	173	172	8,70	5,1%
113	110	103	107	120	113	111	5,83	5,3%
63	57	50	50	57	60	56	5,27	9,4%
53	40	43	50	47	43	46	4,90	10,6%
23	27	27	23	7	27	22	7,76	34,8%

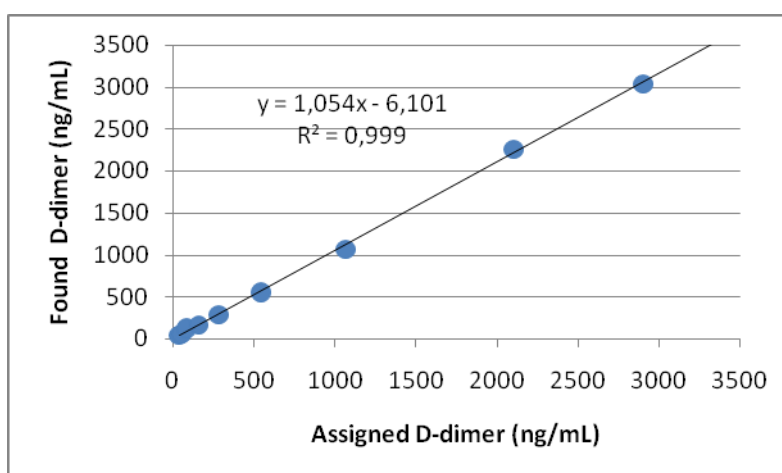
High-dose response

A dilution series of seventeen samples, with a wide range of D-dimer concentrations, was prepared. These samples were assayed on the Sysmex CA-1500 (Table 3, Figure 2). In the range 50 – 3400 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 *Range Over* or *No Linearity*. This prevents high samples being falsely reported within the reportable range. In other words, there is no prozone effect below 130 000 ng/mL.

Table 3: High-dose response

D-dimer (ng/mL)	
Assigned	Found
40	47
56	60
89	110
155	170
286	291
548	544
1068	1068
2108	2259
2907	3038
3416	3611
3767	Range Over
4167	Range Over
8282	Range Over
16463	No Linearity
32601	No Linearity
65101	No Linearity
129518	No Linearity

Figure 2: Found D-dimer values versus assigned



Precision

The precision of MRX143 on Sysmex CA-1500/7000 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 3.5% in the lower range, and 1.9% 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			
	329	311	316	304	312	315	318	runs	n = 7	
	326	305	313	307	324	276	300	replicates	k = 6	
	320	305	298	310	321	303	315			
	323	314	301	289	303	303	303			
	320	296	298	304	315	315	294			
	308	305	298	292	303	315	306	Mean		
Mean	321	306	304	301	313	304	306	308	SD (of mean)= 6.8	
Variance	53	39	68	71	79	234	82	89	Var (of mean)= 46	
SD	7.3	6.2	8.2	8.4	8.9	15.3	9.1	9.5		
CV %	2.3	2.0	2.7	2.8	2.8	5.0	3.0	3.1		
Within run										
Variance		90				Variance	31		Variance	116
SD		9.5				SD	5.5		SD	10.8
CV %		3.1				CV %	1.8		CV %	3.5
dF, n(k-1)		35				dF, n-1	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			
	1602	1572	1545	1524	1538	1571	1550	runs	n = 7	
	1533	1554	1512	1518	1544	1556	1496	replicates	k = 6	
	1575	1590	1512	1527	1547	1550	1526			
	1512	1596	1489	1515	1522	1535	1499			
	1569	1557	1551	1530	1556	1577	1541			
	1545	1527	1548	1545	1550	1538	1463	Mean		
Mean	1556	1566	1526	1527	1544	1554	1512	1541	SD (of mean)= 19.4	
Variance	1058	659	654	112	160	296	1054	570	Var (of mean)= 376	
SD	32.5	25.7	25.6	10.6	12.7	17.2	32.5	23.9		
CV %	2.1	1.6	1.7	0.7	0.8	1.1	2.1	1.6		
Within run										
Variance		570				Variance	281		Variance	813
SD		23.9				SD	16.8		SD	28.5
CV %		1.6				CV %	1.1		CV %	1.9
dF, n(k-1)		35				dF, n-1	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 MRX143 is unaffected by any of the tested Hemoglobin concentrations.

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

Hemoglobin g/L	Replicate 1 ng/mL	Replicate 2 ng/mL	Mean ng/mL	Recovery
0.0	964	922	943	100%
2.0	951	954	953	101%
4.0	967	941	954	101%
6.0	941	938	940	100%
8.0	954	935	945	100%
10.0	935	967	951	101%

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 MRX143 is unaffected by any of the tested Bilirubin concentrations.

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

Bilirubin g/L	Replicate 1 ng/mL	Replicate 2 ng/mL	Mean ng/mL	Recovery
0.0	954	954	954	100%
0.1	999	964	982	103%
0.2	990	970	980	103%
0.3	990	951	971	102%
0.4	973	957	965	101%
0.5	986	970	978	103%

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 MRX143 is only modestly affected by the lipids. With a 10% margin, there is no effect of lipids below 20 g/L.

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

Triglycerides g/L	Replicate 1 ng/mL	Replicate 2 ng/mL	Mean ng/mL	Recovery
1.0	851	900	876	100%
2.9	884	874	879	100%
4.0	884	874	879	100%
7.0	877	858	868	99%
9.4	842	855	849	97%
12.0	864	842	853	97%
14.9	816	851	834	95%

17.7	835	803	819	94%
19.2	806	822	814	93%
21.9	800	791	796	91%

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 MRX143 is unaffected 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	Mean ng/mL	Recovery
0	938	957	948	100%
1	961	929	945	100%
10	935	941	938	99%
100	977	951	964	102%

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 MRX143 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	Mean ng/mL	Recovery
0	954	922	938	100%
1	951	912	932	99%
10	941	941	941	100%
100	945	935	940	100%

Correlation

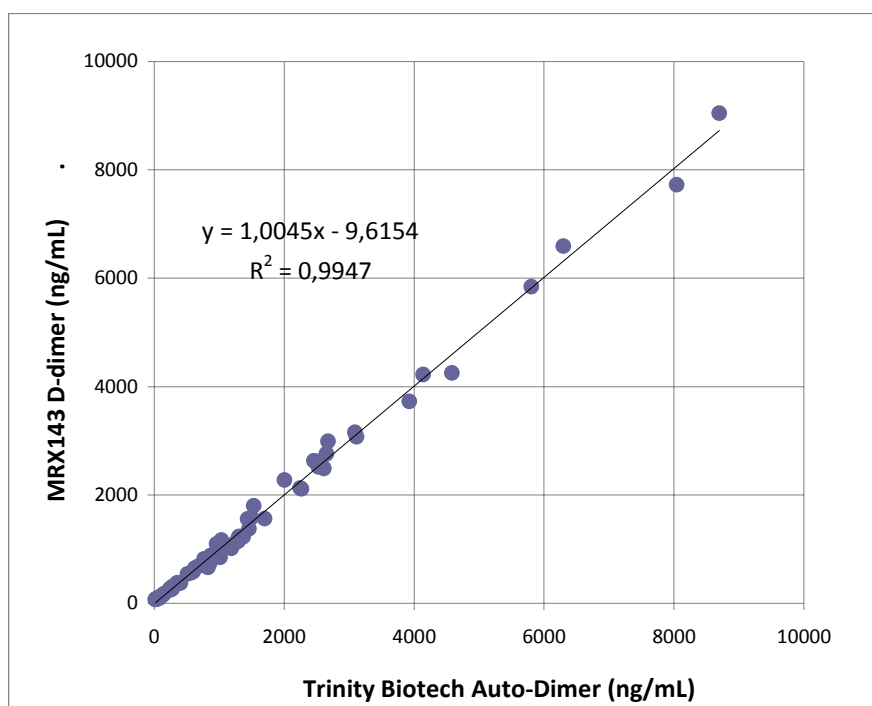
Samples from 66 patients (referred for D-dimer testing to the Umeå University Hospital) were assayed in parallel with two different D-dimer kits on the Sysmex CA-1500: MediRox MRX143 (lot # 00208182) and Trinity Biotech Auto-Dimer (lot # S147053). The data (Table 11, Figure 3) show excellent correlation between the two methods:

$$\text{MRX143} = 1.00 \times \text{Auto-dimer} - 9.6; R^2 = 0.99$$

Table 11: Correlation

Trinity Biotech Auto-Dimer	MediRox MRX143 D-dimer
21	73
21	71
36	85
39	80
50	76
54	95
89	98
96	124
136	156
154	176
243	268
250	256
250	250
257	268
279	306
279	268
357	376
403	371
515	542
567	561
600	586
626	649
669	677
770	819
780	774
811	709
830	658
854	732
870	886
959	1098
1014	847
1035	1169
1057	1085
1190	1015
1252	1119
1281	1144
1296	1147
1300	1219
1307	1231
1365	1237
1368	1228
1440	1554
1460	1375
1499	1578
1531	1799
1699	1561
2008	2276
2249	2126
2267	2112
2459	2631
2527	2517
2545	2617
2582	2533
2613	2489
2650	2761
2675	2987
3089	3157
3114	3072
3928	3726
4138	4224
4584	4252
5806	5846
6300	6594
8043	7722
8700	9044

Figure 3: Correlation MRX143 versus Auto-Dimer



Performance - instrument unrelated

Specificity

For a detailed study of the specificity of the monoclonal antibody in MRX143, please confer separate Technical Report.

Stability

Please confer separate Technical Report, for a detailed account of the MRX143 shelf-life and in-use stability.