

Evaluation Report

Eurolyser Fibrinogen Test Kit (VT0120) on Solo Analyser ("new" format without sample pre-dilution)

Location

Location: Eurolyser Diagnostica GmbH
Operator: Michael Gruber
Date: June - August 2015

Specimens

The specimens used for analysis were taken from multiple sites and were frozen equine lithium-heparin and citrated plasma samples.

Equipment

- Eurolyser solo analyser: Bc14580, Bc14581
- Test kit LOT fibrinogen: 0815-1



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1. Introduction and scope

The Eurolyser solo fibrinogen assay was modified in order to reduce the pre-dilution step (sample predilution)

For this reason the antibody concentration within the R2 cap was increased to make sure that there is no prozone effect at least up to sample concentrations of 800 mg/dl.

2. Comparison Studies

The comparison study is based on the correlation between the results of the Eurolyser fibrinogen assay and the COBAS8000 c701 with fibrinogen reagent from DAKO:
(article codes Q012205 + S200630 + S200530 buffer/antibody/calibrator)

The COBAS 8000 Analyzer was calibrated with a 5 point calibration against 0.9% NaCL and the DAKO fibrinogen calibrator.

The solo fibrinogen assay was calibrated against a commercial available "human" fibrinogen standard with a concentration of 440 mg/dl.

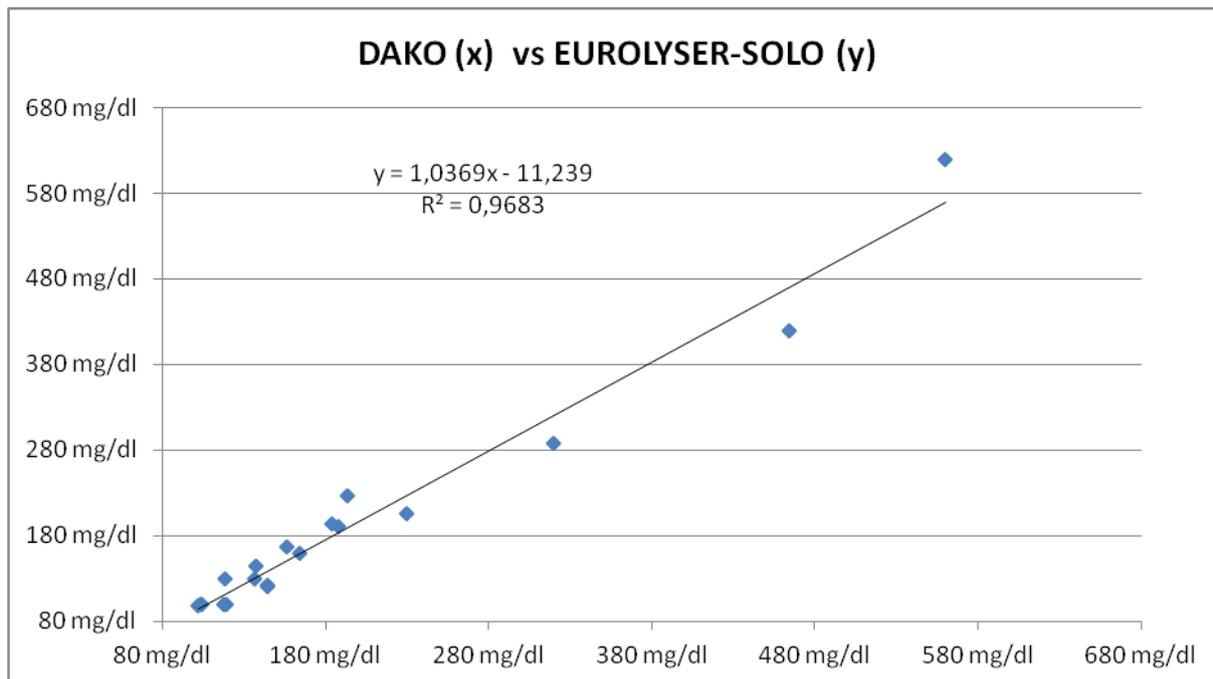
To set a higher calibration point the sample amount of the calibrator was doubled and so the linearity range of the calibration was extrapolated up to 880 mg/dl.

19 patient samples (lithium-heparin plasma and citrated plasma), have been tested.

The acceptance criteria for this comparison study is a coefficient of determination **$R^2 > 0.85$** obtained from linear regression between the Eurolyser solo fibrinogen and DAKO fibrinogen. Target slope (regression fit) in a range of 0.80 - 1.20.

No.	Sample type	Finding No.	Species	DAKO fibrinogen (150-330 mg/dl) COBAS DAKO	FIB Eurolyser (150-330 mg/dl) EUROLYSER solo
1	Lithium-heparin	1506S43044	Horse	119 mg/dl	99 mg/dl
2	Lithium-heparin	1506S43045	Horse	137 mg/dl	144 mg/dl
3	Citrated plasma	1506S43046	Horse	136 mg/dl	129 mg/dl
4	Citrated plasma	1506S43053	Horse	193 mg/dl	226 mg/dl
5	Citrated plasma	1506S43054	Horse	144 mg/dl	122 mg/dl
6	Lithium-heparin	1506S45317	Horse	118 mg/dl	129 mg/dl
7	Lithium-heparin	1506S47320	Horse	156 mg/dl	167 mg/dl
8	Citrated plasma	1506S47321	Horse	144 mg/dl	120 mg/dl
9	Citrated plasma	1506S51129	Horse	188 mg/dl	190 mg/dl
10	Lithium-heparin	1506S51868	Horse	118 mg/dl	100 mg/dl
11	Lithium-heparin	1506S55527	Horse	102 mg/dl	98 mg/dl
12	Citrated plasma	1506S56503	Horse	184 mg/dl	193 mg/dl
13	Citrated plasma	1506S56825	Horse	104 mg/dl	100 mg/dl
14	Citrated plasma	1506S58547	Horse	164 mg/dl	159 mg/dl
15	Lithium-heparin	1506S60380	Horse	104 mg/dl	99 mg/dl
16	Lithium-heparin	1506S61049	Horse	230 mg/dl	205 mg/dl
17	Lithium-heparin	1 UK	UK samples (horse)	560 mg/dl	620 mg/dl
18	Citrated plasma	2 UK	UK samples (horse)	320 mg/dl	288 mg/dl
19	Citrated plasma	3 UK	UK samples (horse)	464 mg/dl	420 mg/dl

Table1: Comparison / Measurements



Equine sample correlation:

The result for the correlation between Eurolyser fibrinogen and COBAS/DAKO fibrinogen is the linear regression function y (solo FIB) = 1.0369 x (COBAS DAKO FIB) – 11.239 and a **$R^2 = 0.9683$** .

3. Imprecision “within-run”

The imprecision “within-run” of Eurolyser’s fibrinogen has been obtained through 20 measurements of 2 different levels using a low control and a real patient sample (high).

No	Control low (target 340 mg/dl)	Sample 17 (620 mg/dl)
1	330 mg/dl	613 mg/dl
2	329 mg/dl	600 mg/dl
3	344 mg/dl	580 mg/dl
4	330 mg/dl	550 mg/dl
5	349 mg/dl	602 mg/dl
6	337 mg/dl	610 mg/dl
7	329 mg/dl	599 mg/dl
8	333 mg/dl	577 mg/dl
9	339 mg/dl	660 mg/dl
10	344 mg/dl	599 mg/dl
11	308 mg/dl	580 mg/dl
12	331 mg/dl	621 mg/dl
13	339 mg/dl	570 mg/dl
14	345 mg/dl	605 mg/dl
15	340 mg/dl	565 mg/dl
16	343 mg/dl	534 mg/dl
17	349 mg/dl	599 mg/dl
18	348 mg/dl	595 mg/dl
19	348 mg/dl	590 mg/dl
20	344 mg/dl	592 mg/dl
mean	338 mg/dl	592 mg/dl
Stabwn	9.99	26.73
cv	2.96%	4.52%

As degree of the imprecision „within-run“, the percentage of the coefficient of variation is:

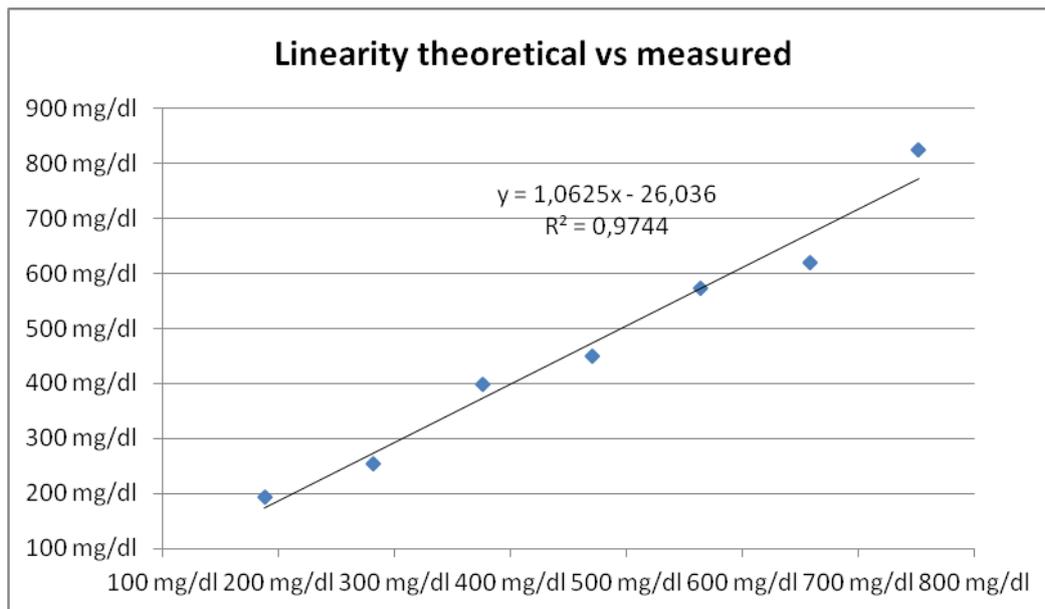
2.96 % at 338 mg/dl

4.52 % at 592 mg/dl

4. Linearity Study

A sample with a concentration of 188mg/dl was used to obtain linearity data. The acceptance criteria is a linear regression with a coefficient of determination $R^2 > 0.9$ and a recovery of **90 - 110 %**.

Sample 1506S51129	DAKO Theoretical value	EUROLYSER Measured value	Recovery
Sample volume x1	188 mg/dl	193 mg/dl	103%
Sample volume x1.50	282 mg/dl	255 mg/dl	90%
Sample volume x2	376 mg/dl	399 mg/dl	106%
Sample volume x2.5	470 mg/dl	450 mg/dl	96%
Sample volume x3	564 mg/dl	573 mg/dl	102%
Sample volume x3.5	658 mg/dl	620 mg/dl	94%
Sample volume x4	752 mg/dl	824 mg/dl	110%



The result for the correlation between theoretical and recovered (measured) values for Eurolyser FIB is the linear regression function y (measured) = 1.0625 x (theoretical) – 26.036 and a $R^2 = 0.9744$.

5. Interferences of the Eurolyser FIB Assay

5.1 Haemoglobin

300 µl sample is mixed with 300 µl haemoglobin with concentrations (0, 200, 300, 400, 500, 1000 mg/dl). The mixtures were measured for fibrinogen on the solo analyser.

As shown in the table, haemoglobin has no effect on the immunturbidimetric determination of fibrinogen.

Haemoglobin mg/dl	Fibrinogen mg/dl	% Variation
0	222.6	-
100	226.7	+ 1.8
200	229.8	+ 3.2
300	227.2	+ 2.1
400	226.6	+ 1.8
500	214.4	- 3.7

5.2 Bilirubin

300 µl sample is mixed with 300 µl of Bilirubin with concentrations (0, 12, 18, 24, 30, 60 mg/dl). The mixtures were measured for fibrinogen on the solo analyser.

As shown in the table Bilirubin has no effect on the immunoturbidimetric determination of fibrinogen.

Bilirubin mg/dl	Fibrinogen mg/dl	% Variation
0	275.3	-
6	282.1	+ 2.5
12	271.9	- 1.2
18	278.0	+ 0.9
24	276.9	+ 0.6
30	281.2	+ 2.1

5.3 EDTA

300 µl sample is mixed with 300 µl of EDTA with concentrations (0, 0.6, 1.2, 2.5, 5, 10 mg/ml). The mixtures were measured for fibrinogen on the solo analyser.

As shown in the table EDTA has no effect on the immunoturbidimetric determination of fibrinogen.

EDTA mg/ml	Fibrinogen mg/dl	% Variation
0	222.6	-
0.6	210.1	- 5.6
1.2	209.0	- 6.1
2.5	216.5	- 2.7
5	212.4	- 4.6
10	215.2	- 3.3

5.4 Natrium Citrate

300 µl sample is mixed with 300 µl of natrium citrate with concentrations (0, 250, 500, 1000, 2000 mg/dl). The mixtures were measured for fibrinogen on the solo analyser.

As shown in the table, natrium citrate has no effect on the immunoturbidimetric determination of fibrinogen.

Natrium citrate mg/dl	Fibrinogen mg/dl	% Variation
0	275.3	-
125	281.2	+ 2.1
250	276.8	+ 0.5
500	261.5	- 5.0
1000	277.3	+ 0.7

5.5 Triglyceride

300 µl sample is mixed with 300 µl of triglyceride with concentrations (0, 156, 312, 625, 1250, 2500, 5000 mg/dl). The mixtures were measured for fibrinogen on the solo analyser.

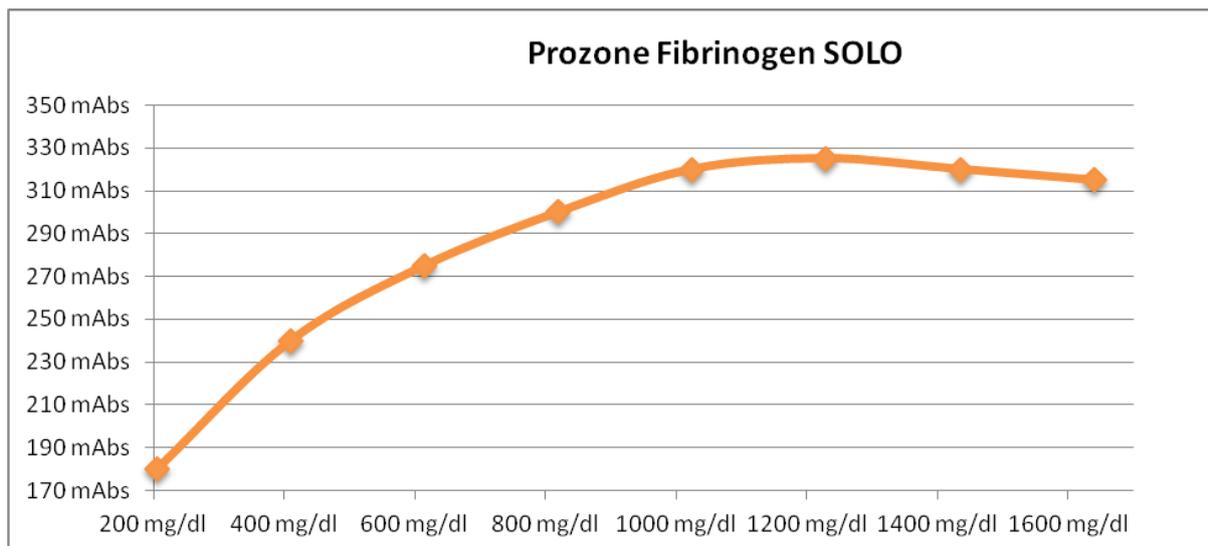
As shown in the table, triglyceride has no effect on the immunoturbidimetric determination of fibrinogen.

Triglyceride mg/dl	FIB mg/dl	% Variation
0	275.3	-
78	270.5	- 1.7
156	285.6	+ 3.7
312	280.1	+ 1.7
625	284.5	+ 3.3
1250	293.5	+ 6.6
2500	267.0	- 3.0

6. Prozone Effect

The prozone effect was tested using a patient sample with known values and measuring the absorption of the solo photometer for several multiplied sample volumes.

Sample volume	Lithium heparin Conc (theoretical)	Equine mAbs SOLO
5 µl	205 mg/dl	180 mAbs
10 µl	410 mg/dl	240 mAbs
15 µl	615 mg/dl	275 mAbs
20 µl	820 mg/dl	300 mAbs
25 µl	1025 mg/dl	320 mAbs
30 µl	1230 mg/dl	325 mAbs
35 µl	1435 mg/dl	320 mAbs
40 µl	1640 mg/dl	315 mAbs



There is no prozone effect up to a level of 950 mg/dl.

It will be specified up to 800mg/dl in order to achieve a security zone for the Prozone Effect

7. Normal Values

As the correlation (see section 2. COMPARISON STUDY) fits perfectly to the COBAS DAKO reference, the normal values have been set the same as on the DAKO FIB for equine samples.

The equine normal range is set as: 150 - 330 mg/dl

Nevertheless, it is recommended that each laboratory establishes its own reference range.