Evaluation Report

Eurolyser PT (INR) test kit (ST0180) on CUBE and smart analysers

Locations

Location 1: Eurolyser Diagnostica GmbH
Operator: Simone Wieser
Date: 30.06.2015

Specimens

The specimens used for analysis were taken from multiple sites and were fresh human citrated blood and plasma samples.

Equipment

- Eurolyser smart analyser:	Bc14261	Bc14262
- Eurolyser CUBE analyser:	Cb 12758	Cb 12759
- Testkit INR	ST0180:	LOT 0615-1

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1. Introduction and Scope

Eurolyser's PT (INR) test kit is used for the determination of prothrombin complex-activity in whole blood according to Owren. It is ideal for monitoring of oral anticoagulation with vitamin-K inhibitors like Warfarin or Marcumar. PT according to Owren measures the combined activity of the vitamin-K dependent coagulation factors II, VII and X. In analysis, the sample is mixed with thromboplastin, CaCl2, fibrinogen and coagulation factor V. The clotting time is prolonged on the therapeutical level (< 0.4 IU/ml) of factor II, V and X in the sample, but the assay is insensitive to heparin in therapeutical levels up to 1.0 IE/ml.

Principle:
Photometrical clot detection at 700 nm

2. Comparison Studies

2.1 Whole Blood (Eurolyser) vs Plasma (ACL TOP)

The comparison study is based on the correlation between the results of the Eurolyser PT INR and the ACL® Top 300 Analyser (ACL Top 300 is a product of Instrumentation Laboratories, a Werfen Company).

49 samples have been analysed in citrate whole blood on CUBE and smart analysers; After a spinning process the plasma of the same samples has been tested on the ACL Top 300 system.

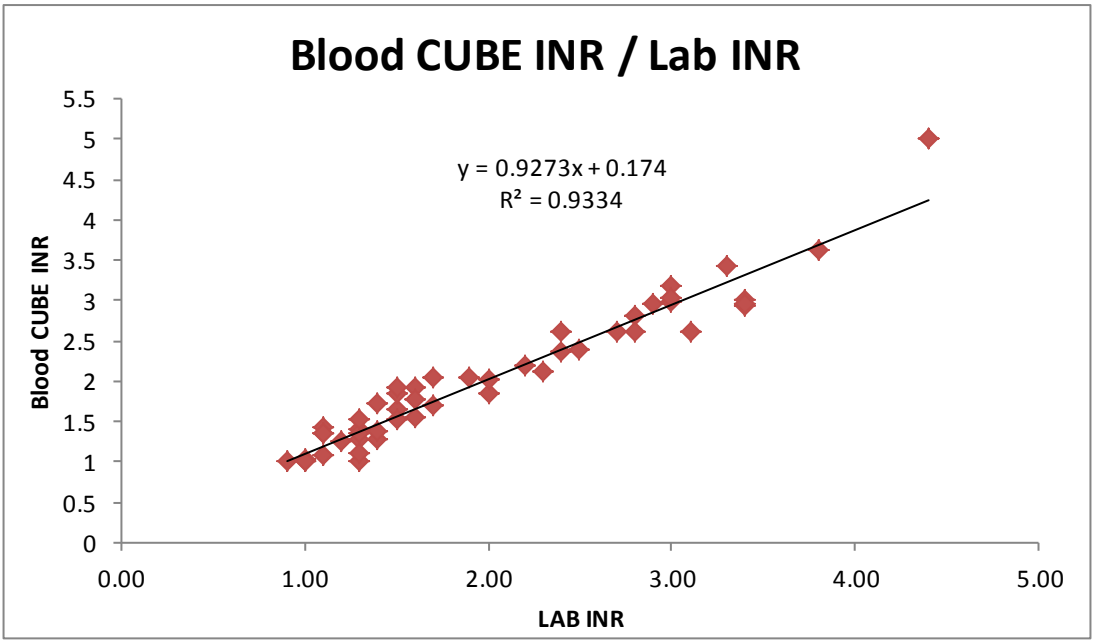
The acceptance criterion for this comparison study is a coefficient of determination **R^2 > 0.90** obtained from linear regression between the Eurolyser INR and the ACL Top 300 INR.

Accepted Slope k = 0.9 – 1.1
Accepted Offset d = +/- 0.2

Sample N°	LAB INR ACL Top 300	Eurolyser (CUBE/smart) Blood INR	Diff ACL-Eurolyser
1	0.90	1.00	- 0.10
2	1.00	1.01	- 0.01
3	1.00	1.02	- 0.02
4	1.00	1.01	- 0.01
5	1.10	1.42	- 0.32
6	1.10	1.34	- 0.24
7	1.10	1.34	- 0.24
8	1.10	1.07	0.03
9	1.20	1.26	- 0.06
10	1.30	1.00	0.30
11	1.30	1.40	- 0.10
12	1.30	1.28	0.02
13	1.30	1.35	- 0.05
14	1.30	1.53	- 0.23
15	1.30	1.10	0.20
16	1.40	1.28	0.12
17	1.40	1.72	- 0.32

18	1.40	1.38	0.02
19	1.50	1.65	- 0.15
20	1.50	1.84	- 0.34
21	1.50	1.93	- 0.43
22	1.50	1.53	- 0.03
23	1.60	1.91	- 0.31
24	1.60	1.78	- 0.18
25	1.60	1.55	0.05
26	1.70	2.05	- 0.35
27	1.70	1.69	0.01
28	1.90	2.04	- 0.14
29	2.00	1.84	0.16
30	2.00	2.03	- 0.03
31	2.20	2.20	0.00
32	2.30	2.12	0.18
33	2.40	2.37	0.03
34	2.40	2.62	- 0.22
35	2.50	2.38	0.12
36	2.70	2.61	0.09
37	2.80	2.80	0.00
38	2.80	2.62	0.18
39	2.90	2.96	- 0.06
40	3.00	3.18	- 0.18
41	3.00	2.97	0.03
42	3.00	3.02	- 0.02
43	3.10	2.62	0.48
44	3.30	3.42	- 0.12
45	3.40	2.94	0.46
46	3.40	3.01	0.39
47	3.40	2.95	0.45
48	3.80	3.62	0.18
49	4.40	5.01	- 0.61
mean	**2.01**	**2.04**	

Samples were measured alternating between CUBE and smart systems (1 - 4 smart / 5 - 8 CUBE, etc.)



Whole blood sample correlation:
The result for the correlation between ACL Top 300 and Eurolyser is the linear regression function:
y (Eurolyser) = 0.9273 x(ACL TOP) + 0.174 and a **R^2 = 0.9334**.

2.2 Citrated plasma (Eurolyser) vs citrated plasma (ACL TOP)

49 samples have been analysed in citrated plasma on CUBE and smart analysers;
The same samples have been tested on the ACL Top 300 system.

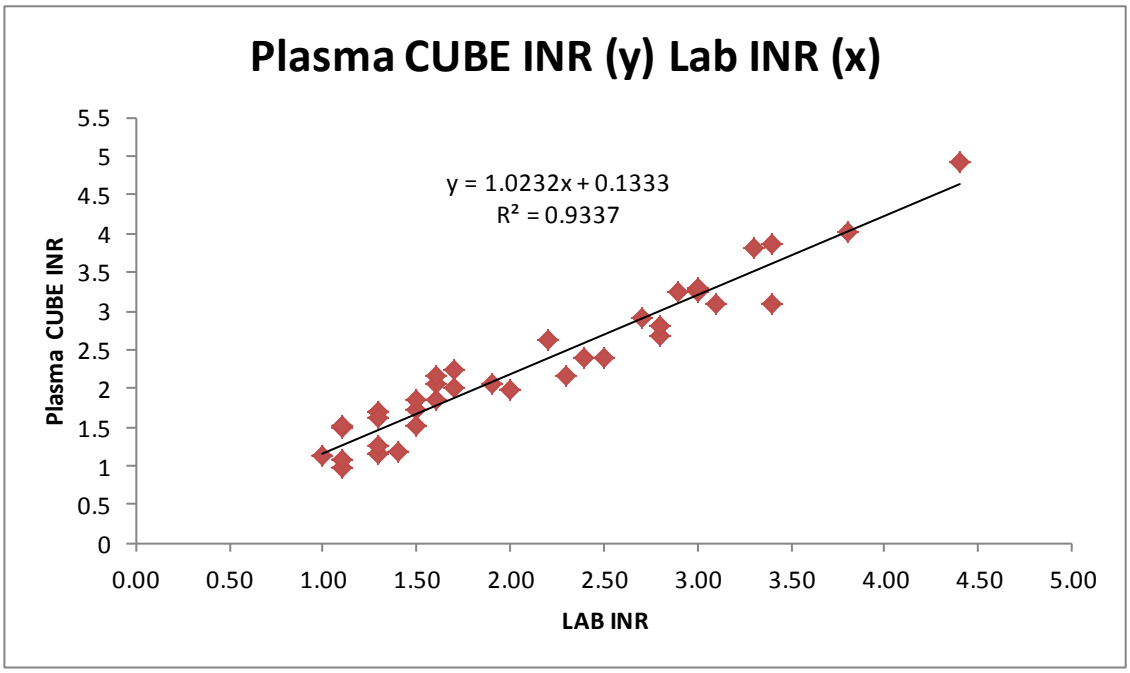
The acceptance criterion for this comparison study is a coefficient of determination **R^2 > 0.90** obtained from linear regression between the Eurolyser INR and the ACL Top 300 INR.

Accepted slope k = 0.9 - 1.1
Accepted offset d = +/- 0.2

Sample N°	LAB INR ACL Top 300	CUBE plasma INR	Diff Lab - Plasma CUBE
1	1.00	1.13	- 0.13
2	1.10	1.50	- 0.40
3	1.10	1.51	- 0.41
4	1.10	0.98	0.12
5	1.10	1.07	0.03
6	1.30	1.16	0.14
7	1.30	1.69	- 0.39
8	1.30	1.15	0.15
9	1.30	1.71	- 0.41
10	1.30	1.63	- 0.33
11	1.30	1.25	0.05
12	1.40	1.19	0.21

13	1.50	1.72	- 0.22
14	1.50	1.52	- 0.02
15	1.50	1.86	- 0.36
16	1.60	2.06	- 0.46
17	1.60	2.15	- 0.55
18	1.60	1.86	- 0.26
19	1.70	2.24	- 0.54
20	1.70	2.02	- 0.32
21	1.90	2.07	- 0.17
22	2.00	1.98	0.02
23	2.20	2.62	- 0.42
24	2.30	2.15	0.15
25	2.40	2.40	0.00
26	2.50	2.40	0.10
27	2.70	2.92	- 0.22
28	2.80	2.68	0.12
29	2.80	2.82	- 0.02
30	2.90	3.25	- 0.35
31	3.00	3.27	- 0.27
32	3.00	3.25	- 0.25
33	3.00	3.30	- 0.30
34	3.10	3.09	0.01
35	3.30	3.82	- 0.52
36	3.40	3.09	0.31
37	3.40	3.87	- 0.47
38	3.80	4.02	- 0.22
39	4.40	4.91	- 0.51
mean	**2.11**	**2.29**	

Samples were measured alternating between CUBE and smart systems (1 – 4 smart / 5 - 8 CUBE, etc.)



Citrated plasma sample correlation:
The result for the correlation between ACL Top 300 and Eurolyser is the linear regression function:
y (Eurolyser) = 1.0232 x(ACL TOP) + 0.1333 and a **R^2 = 0.9337**.

3. Imprecision “day to day”

a. Precision / Reproducibility

In-house precision:
The precision of the Eurolyser INR assay was evaluated according to Clinical and Laboratory Standards Institute (CLSI) EP5-A guideline. In the study, two controls ~ 1.00 INR and ~ 2.20 INR were tested.

Day	**Date**	**Run 1**		**Run 2**		**Mean**			
CUBE	***Control N***	Result 1 (INR)	Result 2 (INR)	Result 1 (INR)	Result 2 (INR)	Run 1 (INR)	Run 2 (INR)	Day (INR)	Day dev.
1	08.06.15	1.04	0.89	0.95	1.04	0.97	1.00	0.98	0.03
2	09.06.15	0.86	0.95	0.96	0.86	0.91	0.91	0.91	- 0.04
3	10.06.15	1.00	1.05	0.85	0.85	1.03	0.85	0.94	- 0.01
4	11.06.15	0.95	1.03	1.03	0.93	0.99	0.98	0.99	0.04
5	12.06.15	1.00	0.88	1.02	0.89	0.94	0.96	0.95	0.00
6	13.06.15	0.88	1.03	0.92	0.93	0.96	0.93	0.94	- 0.01
7	14.06.15	1.02	0.95	1.00	0.95	0.99	0.98	0.98	0.03
8	15.06.15	0.90	0.99	0.93	0.87	0.95	0.90	0.92	- 0.02
9	16.06.15	0.87	0.86	0.99	0.88	0.87	0.94	0.90	- 0.05
10	17.06.15	1.03	0.86	0.95	1.01	0.95	0.98	0.96	0.02
Day	**Date**	**Run 1**		**Run 2**		**Mean**			
smart	***Control AK***	Result 1 (INR)	Result 2 (INR)	Result 1 (INR)	Result 2 (INR)	Run 1 (INR)	Run 2 (INR)	Day (INR)	Day dev.
1	08.06.15	2.24	2.36	2.00	2.20	2.30	2.10	2.20	- 0.01
2	09.06.15	2.00	2.05	2.37	2.36	2.03	2.37	2.20	- 0.02
3	10.06.15	2.34	2.10	2.10	2.18	2.22	2.14	2.18	- 0.03
4	11.06.15	2.25	2.12	2.22	2.00	2.19	2.11	2.15	- 0.06
5	12.06.15	2.32	2.29	2.24	2.21	2.31	2.23	2.27	0.05
6	13.06.15	2.25	2.25	2.25	2.33	2.25	2.29	2.27	0.06
7	14.06.15	2.35	2.35	2.10	2.01	2.35	2.06	2.20	- 0.01
8	15.06.15	2.22	2.37	2.36	2.29	2.30	2.33	2.31	0.10
9	16.06.15	2.37	2.20	2.18	2.17	2.29	2.18	2.23	0.02
10	17.06.15	2.26	2.01	2.05	2.12	2.14	2.09	2.11	- 0.10

Precision	Control N	Control AK			
Data points	40	40			
Mean	0.95 INR	2.21 INR			
Standard dev. (Day-to-Day)	0.03048793	0.05981685			
cv (Day-to-Day) (%)	3.22 %	2.71 %			

Conclusion: For two levels of INR controls reproducibility data showed that the day-to-day imprecision ranged from 3.22 % (0.95 INR) to 2.71 % (2.21 lNR).

4. Within-Run Precision

Citrated whole blood samples and plasma controls have been tested 12 times and the cv value was calculated (1 - 4 tested with CUBE and 5 - 8 tested with smart analysers, etc.):

N°	Citrated whole blood (~ 1.2 INR)	Citrated whole blood (~ 2.6 INR)	Plasma Control (~ 1.0 INR)	Plasma Control (~ 2.3 INR)
1	1.22 INR	2.44 INR	1.04 INR	2.24 INR
2	1.32 INR	2.37 INR	0.86 INR	2.00 INR
3	1.22 INR	2.36 INR	0.92 INR	2.34 INR
4	1.21 INR	2.44 INR	0.95 INR	2.25 INR
5	1.25 INR	2.43 INR	0.96 INR	2.32 INR
6	1.23 INR	2.41 INR	0.85 INR	2.25 INR
7	1.30 INR	2.38 INR	0.93 INR	2.35 INR
8	1.25 INR	2.44 INR	0.89 INR	2.22 INR
9	1.49 INR	2.39 INR	0.92 INR	2.37 INR
10	1.36 INR	2.43 INR	1.00 INR	2.26 INR
11	1.39 INR	2.42 INR	0.93 INR	2.33 INR
12	1.42 INR	2.45 INR	0.95 INR	2.27 INR
mean	1.31 INR	2.41 INR	0.93 INR	2.27 INR
Std dev	0.09 INR	0.03 INR	0.05 INR	0.10 INR
cv	7.05 %	1.28 %	5.75 %	4.30 %

The cv values ranges from 1.28 % (citrated whole blood sample INR 2.41) to 7.05 % (citrated whole blood sample INR 1.31)

5. Interferences in Plasma

The reagent is not affected by bilirubin up to 0.5 g/l, triglycerides up to 10 g/l, haemoglobin up to 10 g/l and heparin* up to 1IE/ml

* *Means unfractionated heparin*