Evaluation Report:

Eurolyser CRP test (ST0100 and ST0102) on

CUBE analyser (CA0100)

Location

Location: Eurolyser Diagnostica GmbH
Operators: Simone Wieser; Franz Helminger; Michael Gruber
Date: July-November 2015 Version 3.0 11.12.2015

Specimens

The specimens used for analysis were taken from multiple sites and were fresh human serum samples as well as EDTA whole blood samples in Sarstedt primary tubes.

Equipment

- Eurolyser CUBE analysers: Cb11857, Cb11858, Cb11859, CB11860
- CRP testkits: LOT0615-1

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Scope and goals of the Evaluation

1. Method comparison

Review of the conformance of the CRP measurement results with the *Eurolyser CUBE*, from whole blood or serum, and the results with the clinical-chemistry fully automated *Abbott Architect ci8200* from serum.

2. Imprecision

Characterization of the precision of the CUBE production model for various CRP concentrations. Both 'within run' and 'day to day precision' were tested.

3. Linearity

Determination of the linearity of the CRP measurement range on the CUBE Analyser for both serum and whole blood samples.

4. Limit of Quantitation (sensitivity LOQ)

Determination of the Limit of Quantitation for both serum and whole blood samples.

5. Prozone (Antigen Excess)

Confirmation of "no Prozone" effect within the linearity range with super-high CRP samples of 64mg/dl (serum) and 60mg/dl (whole blood).

6. Interferences

This trial was conducted to test the common endogenous substance interference.

7. Traceability of CRP Calibration, External QC materials matrix and sample materials

Information on the traceability to IFCC materials and the compatibility with 3rd party EQUAS material.

1. Comparison Studies

The comparison study is based on the correlation between the results of the Eurolyser CUBE Analyser and the ABBOTT® Architect Ci8200®.

The Architect Ci8200 was calibrated with the Abbott CRP calibrator set. The Eurolyser CRP assay was calibrated against a commercial CRP Calibrator from Invicon-Germany and its re-assigned Eurolyser values.

1.1. Comparison Study Serum

113 patient samples (serum from Sarsted primary tubes) were tested.

The acceptance criterion for this comparison study is a coefficient of determination **$R^2 > 0.95$** obtained from linear regression between the Eurolyser and the ABBOTT® Architect© CRP concentrations.

Results are listed in the following table:

CRP Architect ci8200	CRP Eurolyser
0.57 mg/dl	0.45 mg/dl
1.03 mg/dl	0.76 mg/dl
1.51 mg/dl	1.19 mg/dl
3.44 mg/dl	2.62 mg/dl
0.58 mg/dl	0.41 mg/dl
4.94 mg/dl	4.55 mg/dl
0.84 mg/dl	0.66 mg/dl
0.53 mg/dl	0.37 mg/dl
3.37 mg/dl	2.93 mg/dl
2.36 mg/dl	1.82 mg/dl
2.17 mg/dl	1.90 mg/dl
3.09 mg/dl	2.73 mg/dl
0.52 mg/dl	0.33 mg/dl
1.76 mg/dl	1.40 mg/dl
1.36 mg/dl	0.92 mg/dl
3.59 mg/dl	2.94 mg/dl
4.75 mg/dl	4.93 mg/dl
3.07 mg/dl	2.34 mg/dl
2.89 mg/dl	2.43 mg/dl
2.66 mg/dl	2.40 mg/dl

CRP Architect ci8200	CRP Eurolyser
0.88 mg/dl	0.71 mg/dl
0.51 mg/dl	0.34 mg/dl
0.46 mg/dl	0.30 mg/dl
0.51 mg/dl	0.35 mg/dl
0.93 mg/dl	0.77 mg/dl
0.33 mg/dl	0.26 mg/dl
1.7 mg/dl	1.32 mg/dl
3.56 mg/dl	3.33 mg/dl
6.52 mg/dl	6.92 mg/dl
2.27 mg/dl	1.85 mg/dl
0.86 mg/dl	0.57 mg/dl
0.93 mg/dl	0.71 mg/dl
3.45 mg/dl	2.94 mg/dl
1.83 mg/dl	1.45 mg/dl
0.72 mg/dl	0.54 mg/dl
2.13 mg/dl	2.08 mg/dl
0.99 mg/dl	0.66 mg/dl
0.87 mg/dl	0.66 mg/dl
0.88 mg/dl	0.71 mg/dl
0.51 mg/dl	0.34 mg/dl

CRP Architect ci8200	CRP Eurolyser
9.69 mg/dl	10.00 mg/dl
5.91 mg/dl	6.79 mg/dl
0.35 mg/dl	0.25 mg/dl
1.81 mg/dl	1.33 mg/dl
1.68 mg/dl	1.28 mg/dl
0.3 mg/dl	0.22 mg/dl
0.31 mg/dl	0.24 mg/dl
10.91 mg/dl	10.94 mg/dl
7.59 mg/dl	7.84 mg/dl
0.85 mg/dl	0.68 mg/dl
0.28 mg/dl	0.21 mg/dl
2.77 mg/dl	2.44 mg/dl
0.63 mg/dl	0.52 mg/dl
0.38 mg/dl	0.27 mg/dl
0.56 mg/dl	0.40 mg/dl
9.17 mg/dl	8.92 mg/dl
0.54 mg/dl	0.39 mg/dl
1.58 mg/dl	1.19 mg/dl
0.57 mg/dl	0.45 mg/dl
3.62 mg/dl	3.36 mg/dl
0.88 mg/dl	0.63 mg/dl
0.67 mg/dl	0.58 mg/dl
1.3 mg/dl	1.08 mg/dl
8.48 mg/dl	8.54 mg/dl
5.03 mg/dl	5.27 mg/dl
1.7 mg/dl	1.32 mg/dl
3.56 mg/dl	3.33 mg/dl
6.52 mg/dl	6.92 mg/dl
2.27 mg/dl	1.85 mg/dl
0.86 mg/dl	0.57 mg/dl
0.93 mg/dl	0.71 mg/dl
3.45 mg/dl	2.94 mg/dl
1.83 mg/dl	1.45 mg/dl
0.72 mg/dl	0.54 mg/dl
2.13 mg/dl	2.08 mg/dl
0.99 mg/dl	0.66 mg/dl
0.87 mg/dl	0.66 mg/dl

CRP Architect ci8200	CRP Eurolyser
0.46 mg/dl	0.30 mg/dl
0.51 mg/dl	0.35 mg/dl
0.93 mg/dl	0.77 mg/dl
0.33 mg/dl	0.26 mg/dl
1.7 mg/dl	1.31 mg/dl
1.43 mg/dl	1.15 mg/dl
0.96 mg/dl	0.71 mg/dl
0.56 mg/dl	0.39 mg/dl
0.85 mg/dl	0.64 mg/dl
0.74 mg/dl	0.53 mg/dl
0.48 mg/dl	0.33 mg/dl
0.57 mg/dl	0.48 mg/dl
2.49 mg/dl	2.21 mg/dl
1.37 mg/dl	1.19 mg/dl
4.31 mg/dl	4.08 mg/dl
1.63 mg/dl	1.32 mg/dl
0.22 mg/dl	0.17 mg/dl
0.67 mg/dl	0.48 mg/dl
0.84 mg/dl	0.46 mg/dl
1.4 mg/dl	1.07 mg/dl
1.28 mg/dl	0.97 mg/dl
0.42 mg/dl	0.27 mg/dl
0.32 mg/dl	0.21 mg/dl
0.4 mg/dl	0.28 mg/dl
0.39 mg/dl	0.27 mg/dl
0.39 mg/dl	0.25 mg/dl
1.29 mg/dl	0.94 mg/dl
0.45 mg/dl	0.24 mg/dl
1.26 mg/dl	0.96 mg/dl
2.03 mg/dl	1.62 mg/dl
0.24 mg/dl	0.18 mg/dl
1.28 mg/dl	1.01 mg/dl
1.31 mg/dl	0.93 mg/dl
0.7 mg/dl	0.55 mg/dl
0.72 mg/dl	0.50 mg/dl
2.22 mg/dl	1.96 mg/dl

Table 1 Serum Comparison

CRP concentrations in serum obtained with Eurolyser CRP test on Eurolyser CUBE were plotted against CRP concentrations in serum obtained with CRP test on Architect ci8200.

Regression results are summarized in the following chart:

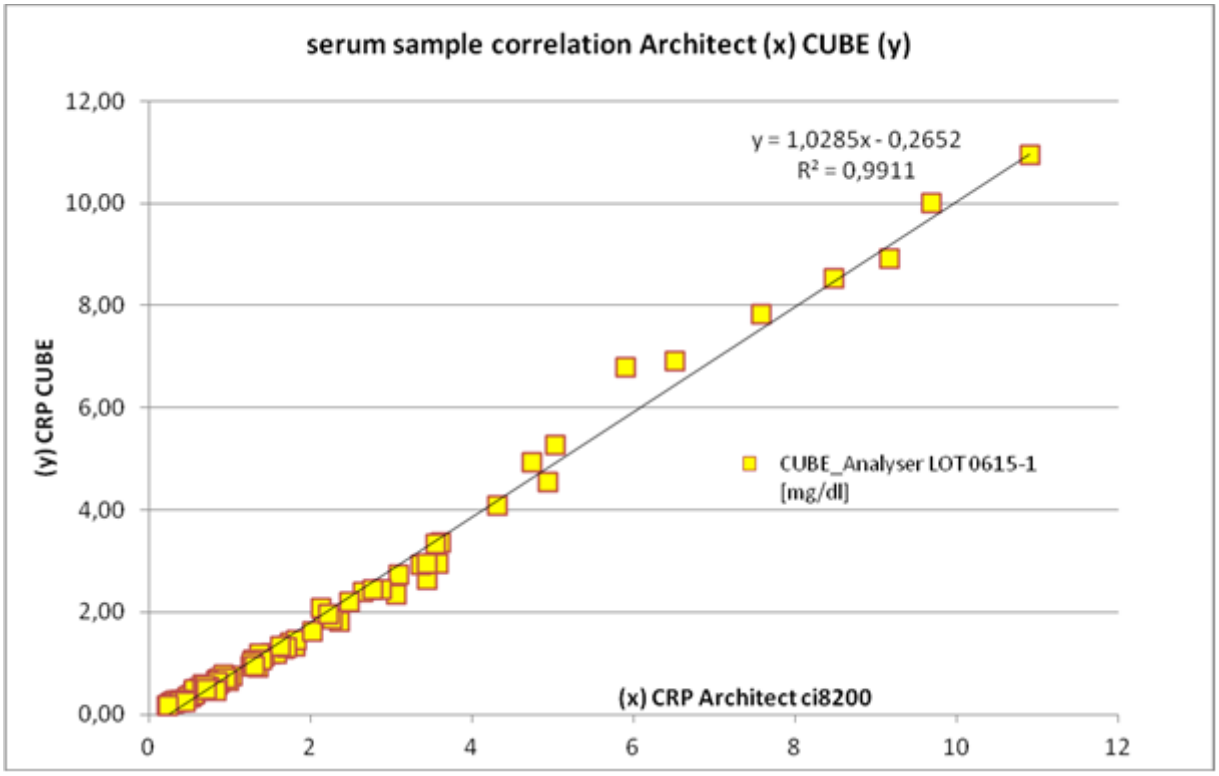

Figure 1 Serum Correlation

The result for the correlation between Eurolyser CUBE CRP and Abbott Architect ci8200 is the linear regression function:

y (CUBE CRP) = 1.0285 x (Abbott CRP) - 0.2652
R^2 = 0.9911.

Conclusion: Method Comparison data showed good correlation and met the method comparison acceptance criteria.

1.2. Comparison Study Whole Blood

113 paired patient samples (whole blood from EDTA K3 tubes and serum from Sarsted primary tubes) were tested.
Whole blood samples were tested on the Eurolyser CUBE. The HCT values were input during sample identification on the Eurolyser CUBE. The corresponding serum sample were tested on the Architect ci8200.

The acceptance criterion for this comparison study is a coefficient of determination **$R^2 > 0.95$** obtained from linear regression between the Eurolyser and the ABBOTT® Architect© CRP concentrations.

Results are listed in the following table:

CRP Architect ci8200	CRP Eurolyser
0.57 mg/dl	0.55 mg/dl
1.03 mg/dl	0.74 mg/dl
1.51 mg/dl	1.15 mg/dl
3.44 mg/dl	2.59 mg/dl
0.58 mg/dl	0.63 mg/dl
4.94 mg/dl	5.66 mg/dl
0.84 mg/dl	0.99 mg/dl
0.53 mg/dl	0.66 mg/dl
3.37 mg/dl	2.89 mg/dl
2.36 mg/dl	1.79 mg/dl
2.17 mg/dl	1.80 mg/dl
3.09 mg/dl	2.66 mg/dl
0.52 mg/dl	0.29 mg/dl
1.76 mg/dl	2.01 mg/dl
1.36 mg/dl	0.89 mg/dl
3.59 mg/dl	2.89 mg/dl
4.75 mg/dl	4.80 mg/dl
3.07 mg/dl	2.29 mg/dl
2.89 mg/dl	3.01 mg/dl
2.66 mg/dl	2.99 mg/dl
9.69 mg/dl	11.90 mg/dl
5.91 mg/dl	6.80 mg/dl
0.35 mg/dl	0.29 mg/dl
1.81 mg/dl	1.40 mg/dl
1.68 mg/dl	1.39 mg/dl
0.3 mg/dl	0.22 mg/dl
0.31 mg/dl	0.24 mg/dl

CRP Architect ci8200	CRP Eurolyser
0.88 mg/dl	0.79 mg/dl
0.51 mg/dl	0.30 mg/dl
0.46 mg/dl	0.29 mg/dl
0.51 mg/dl	0.33 mg/dl
0.93 mg/dl	0.79 mg/dl
0.33 mg/dl	0.27 mg/dl
1.7 mg/dl	1.39 mg/dl
3.56 mg/dl	3.29 mg/dl
6.52 mg/dl	7.09 mg/dl
2.27 mg/dl	1.89 mg/dl
0.86 mg/dl	0.67 mg/dl
0.93 mg/dl	0.70 mg/dl
3.45 mg/dl	2.99 mg/dl
1.83 mg/dl	1.42 mg/dl
0.72 mg/dl	0.59 mg/dl
2.13 mg/dl	2.20 mg/dl
0.99 mg/dl	0.71 mg/dl
0.87 mg/dl	0.72 mg/dl
0.88 mg/dl	0.69 mg/dl
0.51 mg/dl	0.39 mg/dl
0.46 mg/dl	0.33 mg/dl
0.51 mg/dl	0.36 mg/dl
0.93 mg/dl	0.78 mg/dl
0.33 mg/dl	0.27 mg/dl
1.7 mg/dl	1.41 mg/dl
1.43 mg/dl	1.32 mg/dl
0.96 mg/dl	0.78 mg/dl

CRP Architect ci8200	CRP Eurolyser
10.91 mg/dl	11.95 mg/dl
7.59 mg/dl	8.80 mg/dl
0.85 mg/dl	0.70 mg/dl
0.28 mg/dl	0.22 mg/dl
2.77 mg/dl	2.39 mg/dl
0.63 mg/dl	0.51 mg/dl
0.38 mg/dl	0.29 mg/dl
0.56 mg/dl	0.41 mg/dl
9.17 mg/dl	9.70 mg/dl
0.54 mg/dl	0.38 mg/dl
1.58 mg/dl	1.21 mg/dl
0.57 mg/dl	0.46 mg/dl
3.62 mg/dl	3.39 mg/dl
0.88 mg/dl	0.59 mg/dl
0.67 mg/dl	0.61 mg/dl
1.3 mg/dl	1.10 mg/dl
8.48 mg/dl	8.80 mg/dl
5.03 mg/dl	5.31 mg/dl
1.7 mg/dl	1.44 mg/dl
3.56 mg/dl	3.54 mg/dl
6.52 mg/dl	6.80 mg/dl
2.27 mg/dl	1.99 mg/dl
0.86 mg/dl	0.63 mg/dl
0.93 mg/dl	0.69 mg/dl
3.45 mg/dl	2.99 mg/dl
1.83 mg/dl	1.65 mg/dl
0.72 mg/dl	0.89 mg/dl
2.13 mg/dl	2.20 mg/dl
0.99 mg/dl	0.71 mg/dl
0.87 mg/dl	0.67 mg/dl

CRP Architect ci8200	CRP Eurolyser
0.56 mg/dl	0.44 mg/dl
0.85 mg/dl	0.69 mg/dl
0.74 mg/dl	0.55 mg/dl
0.48 mg/dl	0.44 mg/dl
0.57 mg/dl	0.45 mg/dl
2.49 mg/dl	2.08 mg/dl
1.37 mg/dl	1.10 mg/dl
4.31 mg/dl	3.99 mg/dl
1.63 mg/dl	1.55 mg/dl
0.22 mg/dl	0.20 mg/dl
0.67 mg/dl	0.58 mg/dl
0.84 mg/dl	0.49 mg/dl
1.4 mg/dl	1.12 mg/dl
1.28 mg/dl	1.00 mg/dl
0.42 mg/dl	0.30 mg/dl
0.32 mg/dl	0.20 mg/dl
0.4 mg/dl	0.33 mg/dl
0.39 mg/dl	0.20 mg/dl
0.39 mg/dl	0.25 mg/dl
1.29 mg/dl	0.99 mg/dl
0.45 mg/dl	0.39 mg/dl
1.26 mg/dl	0.99 mg/dl
2.03 mg/dl	1.77 mg/dl
0.24 mg/dl	0.20 mg/dl
1.28 mg/dl	1.09 mg/dl
1.31 mg/dl	1.00 mg/dl
0.7 mg/dl	0.59 mg/dl
0.72 mg/dl	0.50 mg/dl
2.22 mg/dl	1.95 mg/dl

Table 2 Serum Whole Blood Comparison

CRP concentrations in whole blood obtained with Eurolyser CRP test on Eurolyser CUBE were plotted against CRP concentrations in serum obtained with CRP test on Architect ci8200.

Regression results are summarized in the following chart:

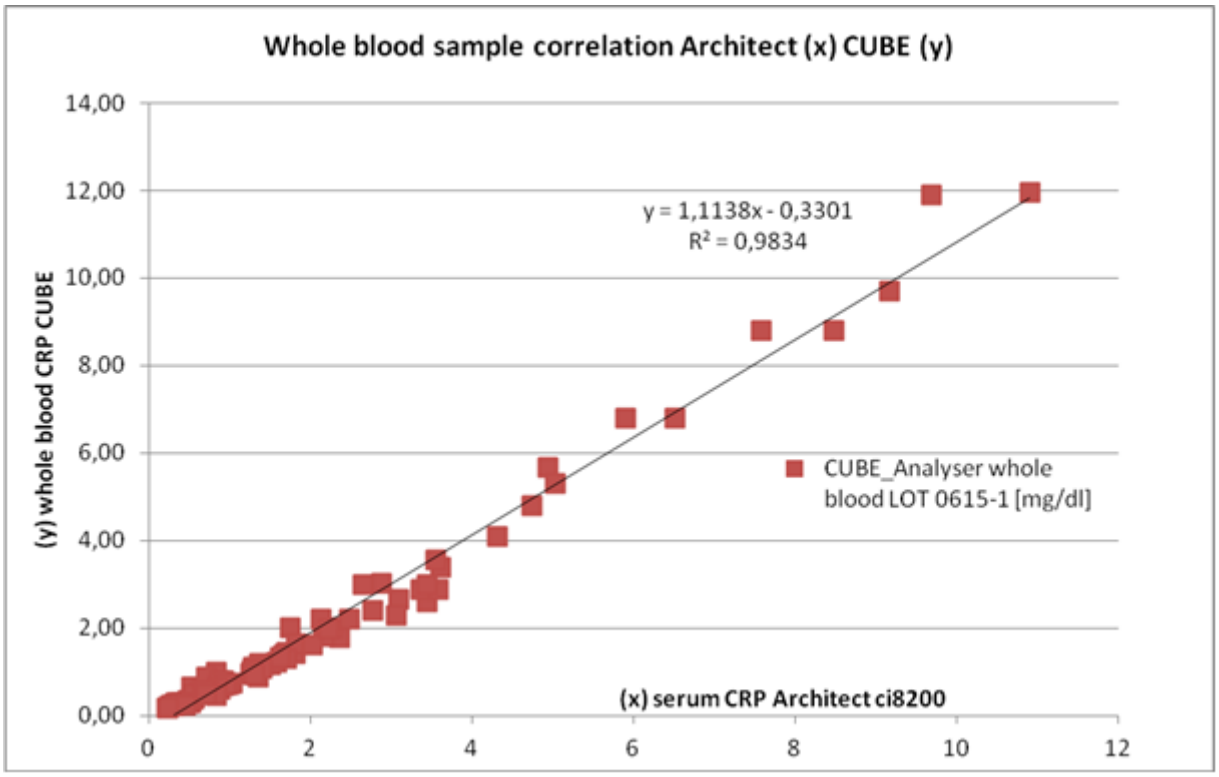

Figure 2 Whole Blood Correlation

The result for the correlation between Eurolyser CUBE CRP whole blood and Abbott Architect ci8200 is the linear regression function:

y (CUBE whole blood CRP) = 1.1138 x (Abbott serum CRP) - 0.3301

R^2 = 0.9834.

Conclusion: Method Comparison data showed good correlation and met the method comparison acceptance criteria.

2. Imprecision

The precision of the Eurolyser CUBE CRP Test was evaluated.

<u>The imprecision is mainly depending on the pipetting accuracy of the operator.</u> The data indicated here have been generated by a moderate complex laboratory trained personnel and reflect a realistic case in daily routine.

2.1. Within Run Imprecision

In the study three serum specimens and three whole blood specimens were tested in 20 runs on four different Eurolyser CUBE Analyzers.

Results are listed in the following table:

Replicate	Serum 1 1.19 mg/dl	Serum 2 4.93 mg/dl	Serum 3 10.0 mg/dl	Blood 1 1.15 mg/dl	Blood 2 4.80 mg/dl	Blood 3 11.9 mg/dl	Analyzer No.
1	1.19 mg/dl	4.89 mg/dl	10.00 mg/dl	1.15 mg/dl	4.81 mg/dl	11.90 mg/dl	Cb11857
2	1.22 mg/dl	5.10 mg/dl	10.50 mg/dl	1.19 mg/dl	5.55 mg/dl	11.30 mg/dl	Cb11858
3	1.08 mg/dl	5.20 mg/dl	11.10 mg/dl	1.10 mg/dl	4.83 mg/dl	11.70 mg/dl	Cb11859
4	1.31 mg/dl	4.77 mg/dl	9.90 mg/dl	1.33 mg/dl	4.88 mg/dl	11.88 mg/dl	Cb11860
5	1.22 mg/dl	4.79 mg/dl	9.80 mg/dl	1.17 mg/dl	5.01 mg/dl	11.67 mg/dl	Cb11857
6	1.15 mg/dl	5.39 mg/dl	10.22 mg/dl	1.13 mg/dl	5.23 mg/dl	11.50 mg/dl	Cb11858
7	1.22 mg/dl	5.24 mg/dl	10.44 mg/dl	1.22 mg/dl	5.47 mg/dl	11.06 mg/dl	Cb11859
8	1.26 mg/dl	5.22 mg/dl	10.59 mg/dl	1.19 mg/dl	4.98 mg/dl	11.44 mg/dl	Cb11860
9	1.19 mg/dl	4.88 mg/dl	9.98 mg/dl	1.22 mg/dl	4.75 mg/dl	10.65 mg/dl	Cb11857
10	1.26 mg/dl	4.78 mg/dl	10.03 mg/dl	1.29 mg/dl	5.07 mg/dl	10.96 mg/dl	Cb11858
11	1.14 mg/dl	5.01 mg/dl	10.22 mg/dl	1.15 mg/dl	5.04 mg/dl	11.88 mg/dl	Cb11859
12	1.21 mg/dl	5.09 mg/dl	10.58 mg/dl	1.17 mg/dl	5.22 mg/dl	11.99 mg/dl	Cb11860
13	1.10 mg/dl	5.01 mg/dl	10.82 mg/dl	1.22 mg/dl	5.17 mg/dl	11.80 mg/dl	Cb11857
14	1.11 mg/dl	4.87 mg/dl	9.83 mg/dl	1.11 mg/dl	4.91 mg/dl	11.90 mg/dl	Cb11858
15	1.22 mg/dl	4.88 mg/dl	9.91 mg/dl	1.07 mg/dl	4.94 mg/dl	11.90 mg/dl	Cb11859
16	1.17 mg/dl	4.79 mg/dl	9.99 mg/dl	1.19 mg/dl	4.88 mg/dl	11.67 mg/dl	Cb11860
17	1.22 mg/dl	4.69 mg/dl	10.01 mg/dl	1.22 mg/dl	5.33 mg/dl	10.26 mg/dl	Cb11857
18	1.16 mg/dl	5.39 mg/dl	10.22 mg/dl	1.16 mg/dl	5.39 mg/dl	11.88 mg/dl	Cb11858
19	1.15 mg/dl	5.41 mg/dl	10.63 mg/dl	1.15 mg/dl	5.45 mg/dl	10.22 mg/dl	Cb11859
20	1.12 mg/dl	5.49 mg/dl	10.22 mg/dl	1.12 mg/dl	5.49 mg/dl	11.33 mg/dl	Cb11860
Mean	**1.19 mg/dl**	**5.04 mg/dl**	**10.25 mg/dl**	**1.18 mg/dl**	**5.12 mg/dl**	**11.44 mg/dl**	
SD	**0.06 mg/dl**	**0.25 mg/dl**	**0.36 mg/dl**	**0.06 mg/dl**	**0.26 mg/dl**	**0.55 mg/dl**	
CV	**5.01%**	**4.94%**	**3.50%**	**5.30%**	**5.00%**	**4.80%**	

Table 3 Eurolyser CRP Within Run Imprecision

Conclusion: For three levels of CRP samples (serum and whole blood) the within-run imprecision was from 3.5% to 5.30%.

2.2. Day to Day Imprecision

In the study two controls were tested in three runs per day over five working days on four different Eurolyser CUBE Analyzers.

Results are listed in the following table:

Replicate	Control Low 1.33 mg/dl	Control High 10.64 mg/dl	Analyser No.
Day 1-1/3	1.33 mg/dl	10.49 mg/dl	Cb11857
Day 1-2/3	1.25 mg/dl	10.61 mg/dl	Cb11858
Day 1-3/3	1.28 mg/dl	9.88 mg/dl	Cb11859
Day 2-1/3	1.41 mg/dl	10.10 mg/dl	Cb11860
Day 2-2/3	1.34 mg/dl	10.14 mg/dl	Cb11857
Day 2-3/3	1.30 mg/dl	10.43 mg/dl	Cb11858
Day 3-1/3	1.24 mg/dl	10.55 mg/dl	Cb11859
Day 3-2/3	1.44 mg/dl	9.99 mg/dl	Cb11860
Day 3-3/3	1.38 mg/dl	10.13 mg/dl	Cb11857
Day 4-1/3	1.33 mg/dl	10.34 mg/dl	Cb11858
Day 4-2/3	1.30 mg/dl	10.44 mg/dl	Cb11859
Day 4-3/3	1.20 mg/dl	10.77 mg/dl	Cb11857
Day 5-1/3	1.21 mg/dl	9.99 mg/dl	Cb11858
Day 5-2/3	1.29 mg/dl	10.66 mg/dl	Cb11859
Day 5-3/3	1.33 mg/dl	11.00 mg/dl	Cb11860
Mean	**1.31 mg/dl**	**10.37 mg/dl**	
SD	**0.07 mg/dl**	**0.32 mg/dl**	
CV	**5.25%**	**3.12%**	

Table 4 Eurolyser CRP Day to Day Imprecision

Conclusion: For two levels of CRP samples (commercial CRP controls) the day to day imprecision was from 3.12% to 5.25%.

3. Linearity Studies

The linearity of the Eurolyser CUBE CRP Test was evaluated.

3.1. Linearity Study Serum

A set of seven levels of linearity materials were prepared by diluting a serum sample containing 12.5 mg/dl of CRP with 0.9% NaCl solultion according to Clinical and Laboratory Standards Institute EP6-A standard and were tested with the Eurolyser CRP test in triplicate on three different Eurolyser CUBE Analyzers.

The levels were prepared according the following list:
Level 1: 0.00 ml 0.9% NaCl solution + 1.00 ml of 12.5 mg/dl CRP
Level 2: 0.25 ml 0.9% NaCl solution + 0.75 ml of 12.5 mg/dl CRP
Level 3: 0.50 ml 0.9% NaCl solution + 0.50 ml of 12.5 mg/dl CRP
Level 4: 0.80 ml 0.9% NaCl solution + 0.20 ml of 12.5 mg/dl CRP
Level 5: 0.90 ml 0.9% NaCl solution + 0.10 ml of 12.5 mg/dl CRP
Level 6: 0.95 ml 0.9% NaCl solution + 0.05 ml of 12.5 mg/dl CRP
Level 7: 0.98 ml 0.9% NaCl solution + 0.02 ml of 12.5 mg/dl CRP

The acceptance criterion for this linearity study is a measured mean value in the range of 90-110% of the expected value.

Results are listed in the following table:

Dilution	Expected Value	Meas 1 Cb11860	Meas 2 Cb11858	Meas 3 Cb11859	Mean	Recovery
Level 1	12.50 mg/dl	12.40 mg/dl	12.10 mg/dl	11.70 mg/dl	12.07 mg/dl	97%
Level 2	9.38 mg/dl	9.01 mg/dl	9.30 mg/dl	8.70 mg/dl	9.00 mg/dl	96%
Level 3	6.25 mg/dl	6.01 mg/dl	5.99 mg/dl	5.55 mg/dl	5.85 mg/dl	94%
Level 4	2.50 mg/dl	2.43 mg/dl	2.66 mg/dl	2.55 mg/dl	2.55 mg/dl	102%
Level 5	1.25 mg/dl	1.32 mg/dl	1.21 mg/dl	1.33 mg/dl	1.29 mg/dl	103%
Level 6	0.63 mg/dl	0.67 mg/dl	0.66 mg/dl	0.74 mg/dl	0.69 mg/dl	110%
Level 7	0.25 mg/dl	0.22 mg/dl	0.28 mg/dl	0.31 mg/dl	0.27 mg/dl	108%

Table 5 Eurolyser CRP Serum Linearity

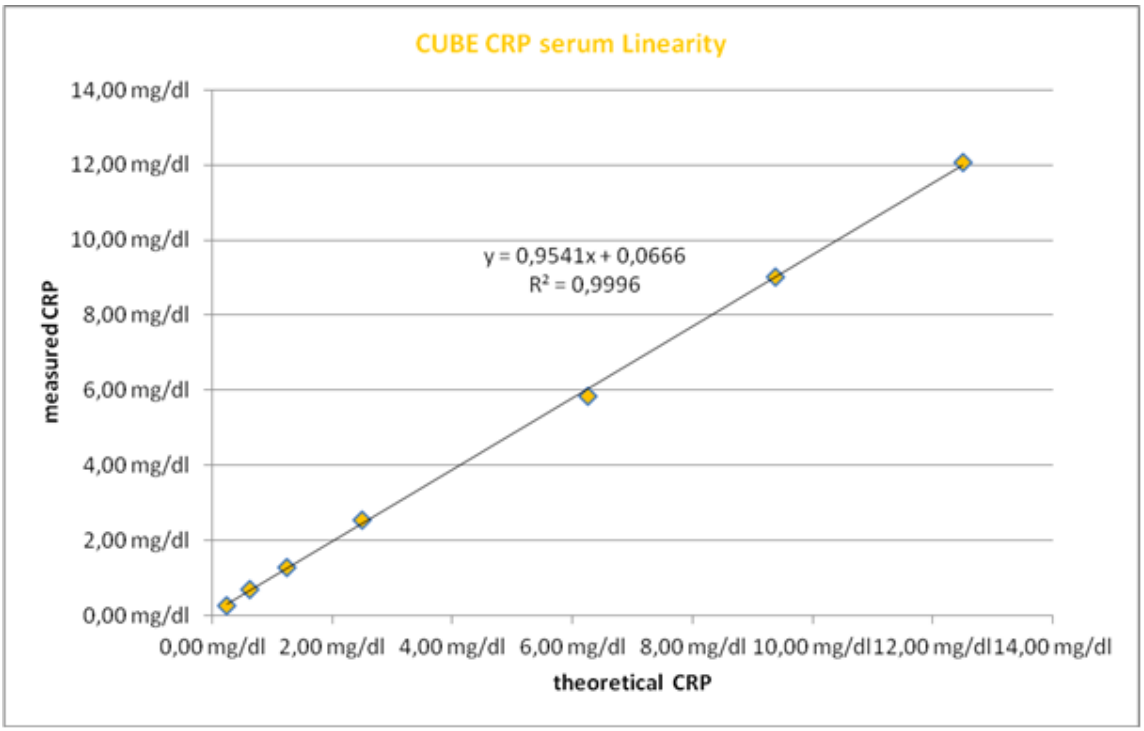

Figure 3 Eurolyser CRP Serum Linearity

Conclusion: The bias between expected and recovered CRP is less than 10% for all levels of the linearity set.

3.2. Linearity Study Whole Blood

A set of seven levels of linearity materials were prepared by the following procedure.
A whole blood sample (EDTA with HCT of 41.5%) containing no CRP was separated in 7 portions. Each portion was centrifuged. A certain amount of plasma was removed and exchanged (spiked) with plasma containing 23.8 mg/dl of CRP.

The levels were prepared according the following list:
Level 1: exchange of 100% of the plasma
Level 2: exchange of 75% of the plasma
Level 3: exchange of 50% of the plasma
Level 4: exchange of 20% of the plasma
Level 5: exchange of 10% of the plasma
Level 6: exchange of 5% of the plasma
Level 7: exchange of 2% of the plasma

The acceptance criterion for this linearity study is a measured mean value in the range of 90-110% of the expected value.

Results are listed in the following table:

Dilution	Expected Value	Meas 1 Cb11858	Meas 2 Cb11860	Meas 3 Cb11859	Mean	Recovery
Level 1	23.80 mg/dl	24.09 mg/dl	23.60 mg/dl	23.00 mg/dl	23.56 mg/dl	99%
Level 2	17.85 mg/dl	17.20 mg/dl	16.10 mg/dl	15.90 mg/dl	16.40 mg/dl	92%
Level 3	11.90 mg/dl	10.80 mg/dl	11.55 mg/dl	10.80 mg/dl	11.05 mg/dl	93%
Level 4	4.76 mg/dl	5.20 mg/dl	4.76 mg/dl	4.88 mg/dl	4.95 mg/dl	104%
Level 5	2.38 mg/dl	2.49 mg/dl	2.76 mg/dl	2.55 mg/dl	2.60 mg/dl	109%
Level 6	1.19 mg/dl	1.23 mg/dl	1.39 mg/dl	1.32 mg/dl	1.31 mg/dl	110%
Level 7	0.48 mg/dl	0.45 mg/dl	0.51 mg/dl	0.59 mg/dl	0.52 mg/dl	109%

Table 6 Eurolyser CRP Whole Blood Linearity

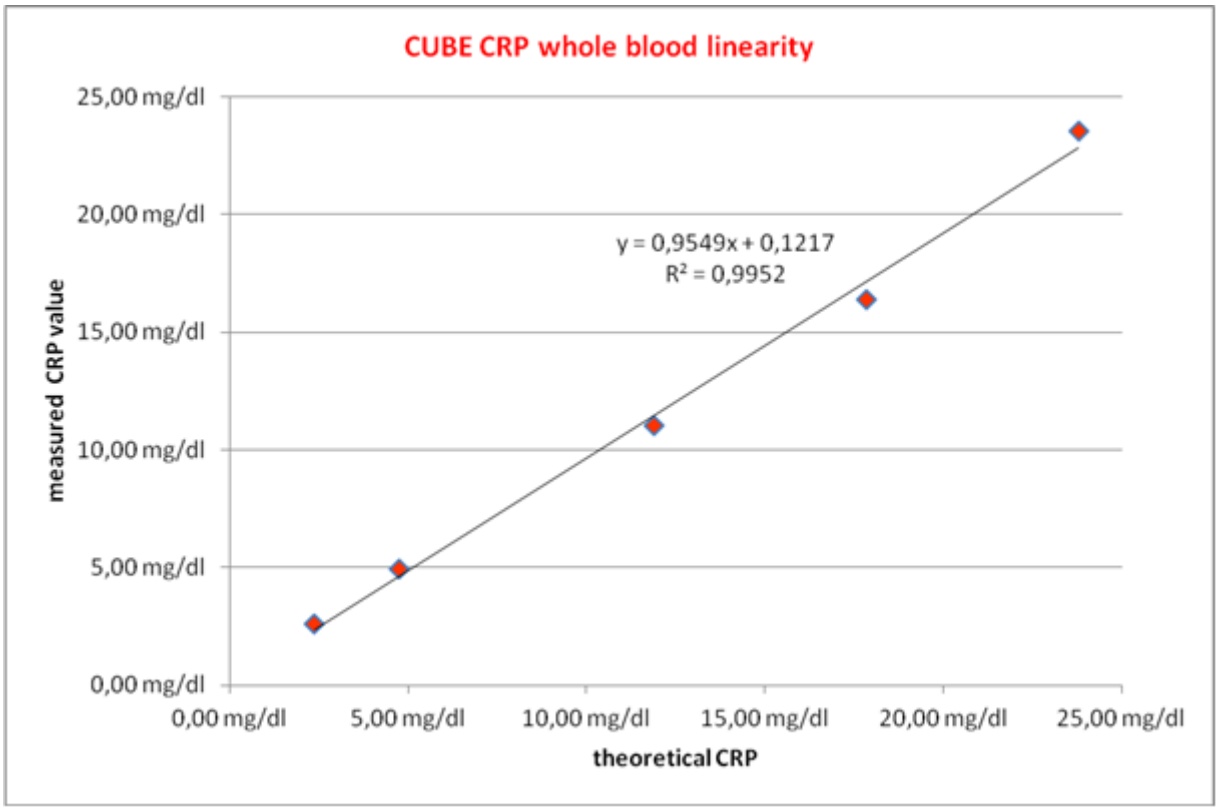

Figure 4 Eurolyser CRP Whole Blood Linearity

Conclusion: The bias between expected and recovered CRP is less than 10% for all levels of the linearity set.

4. Limit of Quantitation (Sensitivity testing)

LOQ (Limit of Quantitation) is determined as the lowest sample run that displayed CV < 20%.

4.1. Limit of Quantitation Serum

To calculate the LOQ for serum three patient serum samples with CRP concentrations of 0.32 mg/dl, 0.15 mg/dl and 0.09 mg/dl were tested with the Eurolyser CRP Test on Eurolyser CUBE analyzers. Each sample was tested 20 times. Regression analysis was used to estimate the LOQ.

Results are listed in the following table:

Replicate	Serum 1 0.32 mg/dl	Serum 2 0.15 mg/dl	Serum 3 0.09mg/dl	Analyser
1	0.32 mg/dl	0.16 mg/dl	0.09 mg/dl	Cb11857
2	0.29 mg/dl	0.14 mg/dl	0.10 mg/dl	Cb11858
3	0.33 mg/dl	0.18 mg/dl	0.11 mg/dl	Cb11859
4	0.34 mg/dl	0.19 mg/dl	0.10 mg/dl	Cb11860
5	0.38 mg/dl	0.14 mg/dl	0.14 mg/dl	Cb11857
6	0.41 mg/dl	0.14 mg/dl	0.10 mg/dl	Cb11858
7	0.36 mg/dl	0.18 mg/dl	0.12 mg/dl	Cb11859
8	0.28 mg/dl	0.22 mg/dl	0.11 mg/dl	Cb11860
9	0.39 mg/dl	0.13 mg/dl	0.11 mg/dl	Cb11857
10	0.40 mg/dl	0.14 mg/dl	0.10 mg/dl	Cb11858
11	0.41 mg/dl	0.18 mg/dl	0.10 mg/dl	Cb11859
12	0.39 mg/dl	0.23 mg/dl	0.09 mg/dl	Cb11860
13	0.44 mg/dl	0.22 mg/dl	0.10 mg/dl	Cb11857
14	0.28 mg/dl	0.13 mg/dl	0.10 mg/dl	Cb11858
15	0.33 mg/dl	0.17 mg/dl	0.12 mg/dl	Cb11859
16	0.35 mg/dl	0.18 mg/dl	0.17 mg/dl	Cb11860
17	0.32 mg/dl	0.16 mg/dl	0.14 mg/dl	Cb11857
18	0.29 mg/dl	0.14 mg/dl	0.10 mg/dl	Cb11858
19	0.33 mg/dl	0.17 mg/dl	0.10 mg/dl	Cb11859
20	0.35 mg/dl	0.19 mg/dl	0.16 mg/dl	Cb11860
Mean	**0.35 mg/dl**	**0.17 mg/dl**	**0.11 mg/dl**	
SD	**0.05**	**0.03**	**0.02**	
CV	**13.47%**	**18.00%**	**19.75%**	

Table 7 Eurolyser CRP Serum LOQ

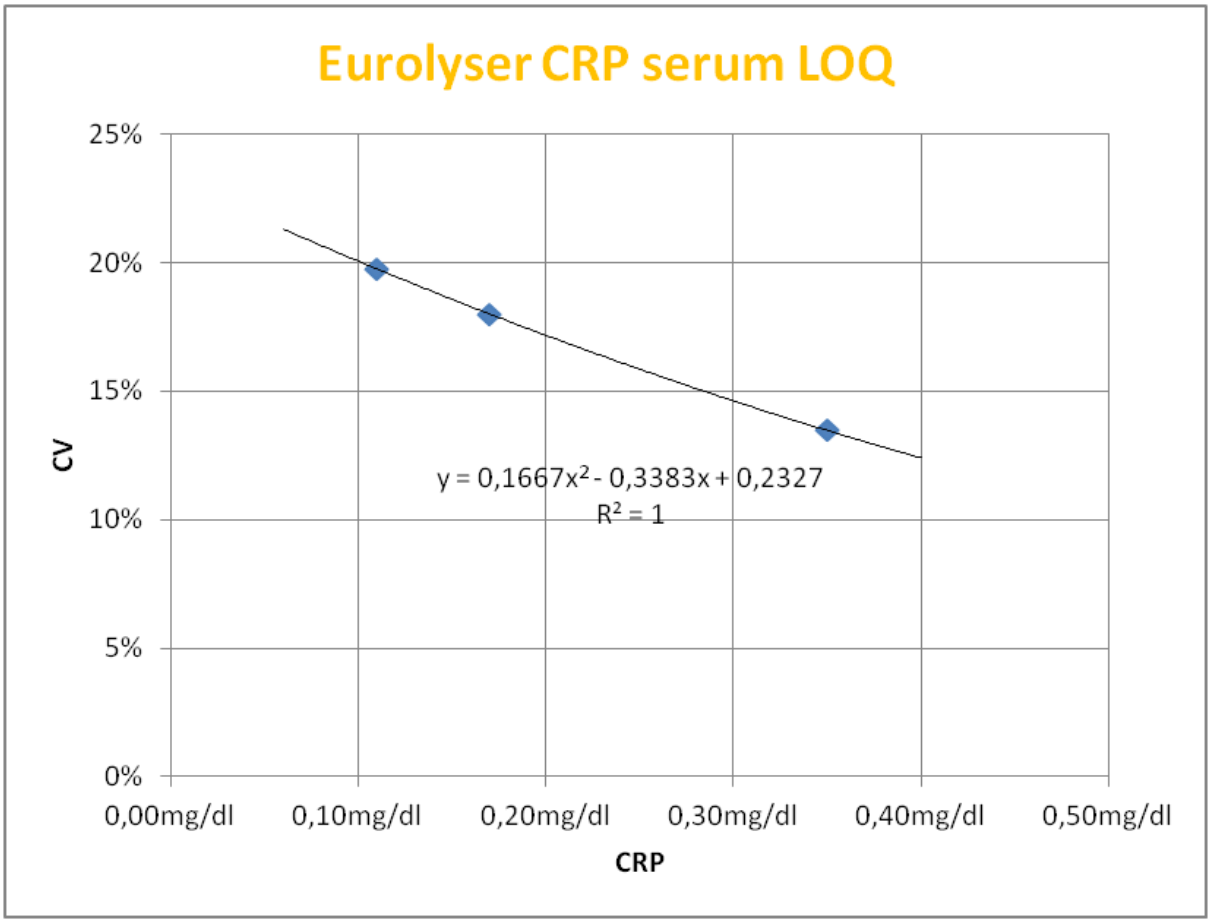

Figure 5 Eurolyser CRP Serum LOQ

Conclusion: The estimated LOQ for serum is 0.10 mg/dl determined by regression analysis.

4.2. Limit of Quantitation Whole Blood

To calculate the LOQ for whole blood three patient EDTA whole blood samples with CRP concentrations of 0.55 mg/dl, 0.43 mg/dl and 0.22 mg/dl were tested with the Eurolyser CRP Test on Eurolyser CUBE analyzers. Each sample was tested 20 times. Regression analysis was used to estimate the LOQ.

Results are listed in the following table:

Replicate	Whole blood 1 0.55 mg/dl	Whole blood 2 0.43 mg/dl	Whole blood 3 0.22 mg/dl	Analyser
1	0.55 mg/dl	0.49 mg/dl	0.21 mg/dl	Cb11857
2	0.53 mg/dl	0.44 mg/dl	0.19 mg/dl	Cb11858
3	0.51 mg/dl	0.39 mg/dl	0.24 mg/dl	Cb11859
4	0.49 mg/dl	0.38 mg/dl	0.22 mg/dl	Cb11860
5	0.48 mg/dl	0.38 mg/dl	0.17 mg/dl	Cb11857
6	0.50 mg/dl	0.41 mg/dl	0.15 mg/dl	Cb11858
7	0.45 mg/dl	0.36 mg/dl	0.18 mg/dl	Cb11859
8	0.41 mg/dl	0.33 mg/dl	0.22 mg/dl	Cb11860
9	0.48 mg/dl	0.39 mg/dl	0.22 mg/dl	Cb11857
10	0.53 mg/dl	0.40 mg/dl	0.19 mg/dl	Cb11858
11	0.58 mg/dl	0.43 mg/dl	0.18 mg/dl	Cb11859
12	0.55 mg/dl	0.39 mg/dl	0.32 mg/dl	Cb11860
13	0.49 mg/dl	0.44 mg/dl	0.22 mg/dl	Cb11857
14	0.47 mg/dl	0.37 mg/dl	0.15 mg/dl	Cb11858
15	0.49 mg/dl	0.36 mg/dl	0.18 mg/dl	Cb11859
16	0.51 mg/dl	0.36 mg/dl	0.18 mg/dl	Cb11860
17	0.55 mg/dl	0.37 mg/dl	0.16 mg/dl	Cb11857
18	0.51 mg/dl	0.35 mg/dl	0.16 mg/dl	Cb11858
19	0.43 mg/dl	0.39 mg/dl	0.20 mg/dl	Cb11859
20	0.55 mg/dl	0.44 mg/dl	0.19 mg/dl	Cb11860
Mean	**0.50 mg/dl**	**0.39 mg/dl**	**0.20 mg/dl**	
SD	**0.04**	**0.04**	**0.04**	
CV	**8.63%**	**9.80%**	**19.69%**	

Table 8 Eurolyser CRP Whole Blood LOQ

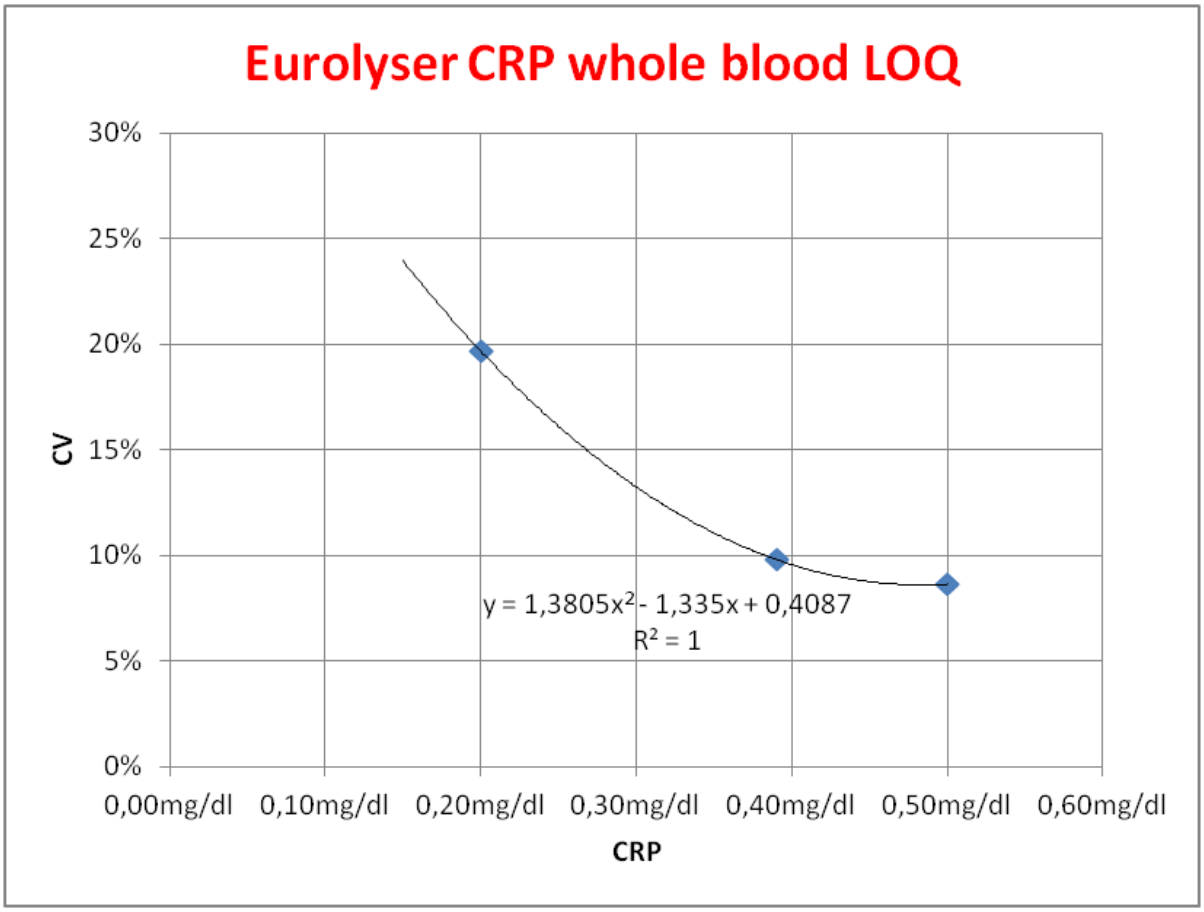

Figure 6 Eurolyser CRP Whole Blood LOQ

Conclusion: The estimated LOQ for whole blood is 0.20 mg/dl determined by regression analysis.

5. Prozone

Confirmation of “no Prozone” effect within the linearity range of the Eurolyser CRP Test.

5.1. Prozone Study Serum

A set of eight levels of materials were prepared from a calibrator set and were tested with the Eurolyser CRP test on a Eurolyser CUBE Analyzer.

The levels were prepared according the following list:
Level 1: Calibrator with 1.0 mg/dl CRP
Level 2: Calibrator with 2.0 mg/dl CRP
Level 3: Calibrator with 4.0 mg/dl CRP
Level 4: Calibrator with 8.0 mg/dl CRP
Level 5: Calibrator with 12.0 mg/dl CRP
Level 6: Calibrator with 16.0 mg/dl CRP
Level 7: Calibrator with 32.0 mg/dl CRP
Level 8: Calibrator with 64.0 mg/dl CRP

The acceptance criterion for this prozone study is that no sample with high CRP is reported lower than the upper limit of the measurement range (12.0 mg/dl).

Results are listed in the following table:

Level	Theoretical Concentration	Measured Concentration
Level 1	1.0 mg/dl	1.10 mg/dl
Level 2	2.0 mg/dl	1.85 mg/dl
Level 3	4.0 mg/dl	4.33 mg/dl
Level 4	8.0 mg/dl	7.80 mg/dl
Level 5	12.0 mg/dl	11.60 mg/dl
Level 6	16.0 mg/dl	14.50 mg/dl
Level 7	32.0 mg/dl	15.50 mg/dl
Level 8	64.0 mg/dl	15.00 mg/dl

Table 9 Eurolyser CRP Serum Prozone

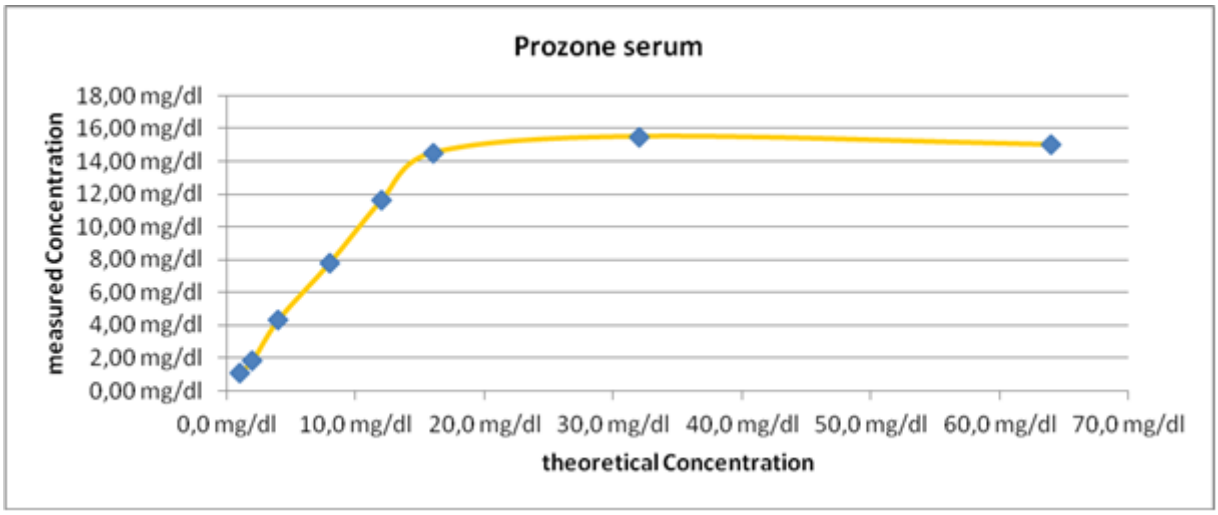

Figure 7 Eurolyser CRP Serum Prozone

Conclusion: There is no prozone effect within the measurement range.

5.2. Prozone Study Whole Blood

A set of eight levels of materials were prepared. For levels 1-5 five patient EDTA whole blood samples with CRP concentrations of 1.1 mg/dl, 2.5 mg/dl, 4.4 mg/dl, 9.6 mg/dl and 11.5 mg/dl were used. For levels 6-8 a whole blood sample (EDTA) containing no CRP was separated in 3 portions. Each portion was centrifuged. A certain amount of plasma was removed and exchanged (spiked) with plasma containing 64.0 mg/dl of CRP. All eight levels were tested with the Eurolyser CRP test on a Eurolyser CUBE Analyzer.

The levels 6-8 were prepared according the following list:
Level 6: exchange of 93.8% of the plasma
Level 7: exchange of 46.9% of the plasma
Level 8: exchange of 23.4% of the plasma

The acceptance criterion for this prozone study is that no sample with high CRP is reported lower than the upper limit of the measurement range (24.0 mg/dl).

Results are listed in the following table:

Level	Theoretical Concentration	Measured Concentration
Level 1	1.1 mg/dl	0.99 mg/dl
Level 2	2.5 mg/dl	2.29 mg/dl
Level 3	4.4 mg/dl	4.39 mg/dl
Level 4	9.6 mg/dl	8.88 mg/dl
Level 5	11.5 mg/dl	11.60 mg/dl
Level 6	15.0 mg/dl	14.70 mg/dl
Level 7	30.0 mg/dl	26.90 mg/dl
Level 8	60.0 mg/dl	25.50 mg/dl

Table 10 Eurolyser CRP Whole Blood Prozone

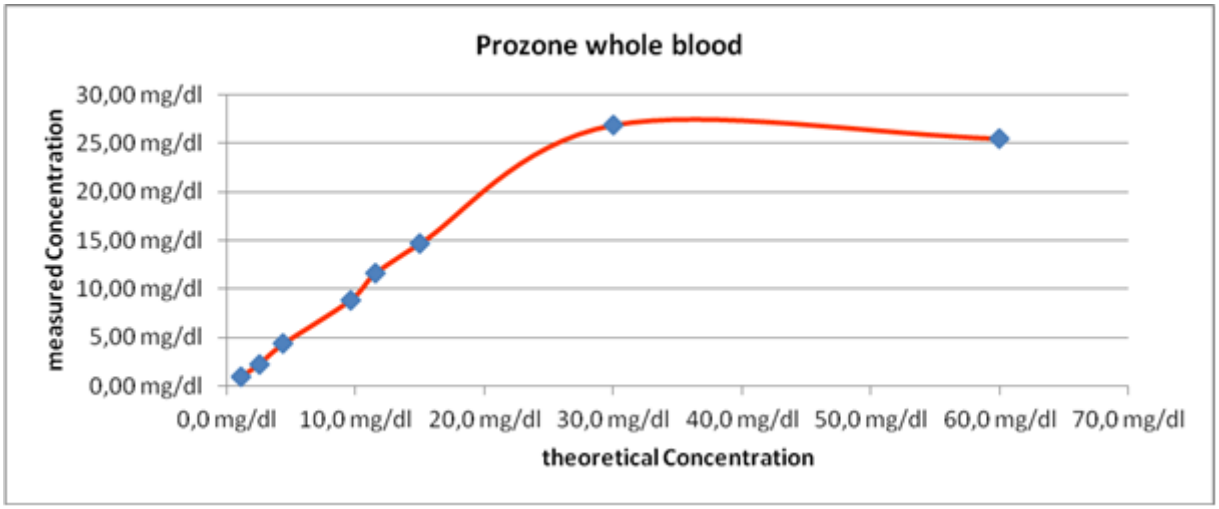

Figure 8 Eurolyser CRP Whole Blood Prozone

Conclusion: There is no prozone effect within the measurement range.

6. Interferences

To determine the level of interference from the substances normally present in whole blood the Eurolyser CRP Test was used to test two whole blood samples with 1.2 mg/dl CRP concentration (Sample 1) and 8.4 mg/dl CRP concentration (Sample 2) spiked with various concentrations of substances following Clinical and Laboratory Standards Institute EP7-A "Interference Testing in Clinical Chemistry: dose-response guidelines".

To ensure a suitable degree of precision, each sample spiked with interference substances was tested in triplicates.

The interference substances examined and their concentrations tested are listed in the following table:

Interference Substance	C1	C2	C3	C4	C5
Ascorbic acid	0 mg/dl	17.6 mg/dl	44 mg/dl	88 mg/dl	176 mg/dl
Ascorbic acid	0 mg/dl	4.4 mg/dl	8.8 mg/dl	10 mg/dl	13.2 mg/dl
Bilirubin	0 mg/dl	10mg/dl	20mg/dl	30mg/dl	40 mg/dl
Conjugated Bilirubin	0 mg/dl	10mg/dl	20mg/dl	30mg/dl	40 mg/dl
Triglycerides	0 mg/dl	250mg/dl	500mg/dl	750mg/dl	1000 mg/dl
Rheumatoid Factor	0 IU/ml	250 IU/ml	500 IU/ml	750 IU/ml	1000 IU/ml

Table 11 Interference Substances

The stock solutions for interference testing were prepared as follows:

Interference Substance	Stock solution	Preparation Method
Ascorbic acid	0.5 M	Dissolve 88mg in 1ml cold water; Keep stock on ice before testing
Ascorbic acid	0.05 M	Dissolve 8.8mg in 1ml cold water; Keep stock on ice before testing
Bilirubin	40 mg/ml	Dissolve 40mg bilirubin unconjugated in 1 ml 0.1N NaOH
Conjugated Bilirubin	40 mg/ml	Dissolve 40mg bilirubin unconjugated in 1 ml water
Triglycerides	1000 mg/ml	Neat solution
RF Positive Serum Control	1500 IU/ml	Prepared from Human Plasma
RF Negative Serum	0 IU/ml	Prepared from Human Plasma

Table 12 Stock Solutions

Results are listed in the following table:

Bilirubin					
	0 mg/dl	**10 mg/dl**	**20 mg/dl**	**30 mg/dl**	**40 mg/dl**
	1.10	1.15	1.18	1.22	1.18
Sample 1 [CRP] mg/dl	1.05	1.11	1.19	1.33	1.29
	1.30	1.33	1.20	1.24	1.29
Mean	1.15	1.20	1.19	1.26	1.25
Recovery %	100%	104.1%	103.5%	109.9	109.0%
	8.80	9.10	9.00	9.20	9.33
Sample 2 [CRP] mg/dl	8.40	8.80	9.10	9.00	9.10
	8.50	8.60	9.30	9.30	9.30
Mean	8.57	8.83	9.13	9.17	9.24
Recovery %	100%	103.1%	106.6%	107.0	107.9%

Conjugated Bilirubin					
	0 mg/dl	**10 mg/dl**	**20 mg/dl**	**30 mg/dl**	**40 mg/dl**
	1.20	1.19	1.22	1.18	1.20
Sample 1 [CRP] mg/dl	1.25	1.21	1.29	1.31	1.33
	1.10	1.08	1.15	1.39	1.37
Mean	1.18	1.16	1.22	1.29	1.30
Recovery %	100%	98.0%	103.1%	109.3%	109.9%
	8.50	8.65	9.10	8.90	9.41
Sample 2 [CRP] mg/dl	8.45	8.79	8.05	8.55	8.99
	8.30	8.73	9.50	9.70	9.25
Mean	8.42	8.72	8.88	9.05	9.22
Recovery %	100%	103.6%	105.5%	107.5%	109.5%

Ascorbic Acid					
	0 mg/dl	**17.6 mg/dl**	**44 mg/dl**	**88 g/dl**	**176 mg/dl**
	8.20	8.30	8.30	8.50	8.80
Sample 2 [CRP] mg/dl	8.40	8.10	8.50	8.66	9.05
	8.30	8.10	8.40	8.44	9.14
Mean	8.30	8.17	8.40	8.53	9.00
Recovery %	100%	98.4%	101.2%	102.8	108.4%
	1.15	1.18	1.23	1.22	1.33
Sample 1 [CRP] mg/dl	1.19	1.16	1.31	1.31	1.25
	1.23	1.10	1.29	1.33	1.19
Mean	1.19	1.15	1.28	1.29	1.26
Recovery %	100%	96.4%	107.3%	108.1%	105.6%

Triglycerides					
	0 mg/dl	**250 mg/dl**	**500 g/dl**	**750 g/dl**	**1000 mg/dl**
	1.18	1.22	1.10	1.18	1.15
Sample 1 [CRP] mg/dl	1.14	1.05	1.19	1.08	1.10
	1.22	1.10	0.99	1.10	0.99
Mean	1.18	1.12	1.09	1.12	1.08
Recovery %	100%	95.2%	92.7%	94.9%	91.5%
	8.30	8.10	7.50	8.11	7.50
Sample 2 [CRP] mg/dl	8.33	8.20	7.80	7.99	7.77
	8.27	8.14	8.10	7.76	7.90
Mean	8.30	8.15	7.80	7.95	7.72
Recovery %	100%	98.2%	94.0%	95.8%	93.1%

RF Rheumatoid Factors					
	0 IU/ml	**250 IU/ml**	**500 IU/ml**	**750 IU/ml**	**1000 IU/ml**
	1.23	1.18	1.29	1.22	1.29
Sample 1 [CRP] mg/dl	1.31	1.09	1.22	1.27	1.33
	1.19	1.19	1.19	1.08	1.22
Mean	1.24	1.15	1.23	1.19	1.16
Recovery %	100%	92.8%	99.2%	95.7%	93.3%
	8.38	8.19	7.99	7.89	7.56
Sample 2 [CRP] mg/dl	8.44	8.33	8.26	7.78	7.88
	8.23	8.14	8.05	8.00	7.93
Mean	8.35	8.19	8.10	7.89	7.79
Recovery %	100%	98.1%	97.0%	94.5%	93.3%

Table 13 Results for Interference Factors

Conclusion: The common interfering substances had no significant interference up to the concentrations provided below.

Interference	Concentration
Triglyceride	1000 mg/dl
Ascorbic Acid	10 mg/dl
Bilirubin	40 mg/dl
Bilirubin Conjugated	40 mg/dl
Rheumatoid Factor	1000 IU/ml

Table 14 Interference Concentrations

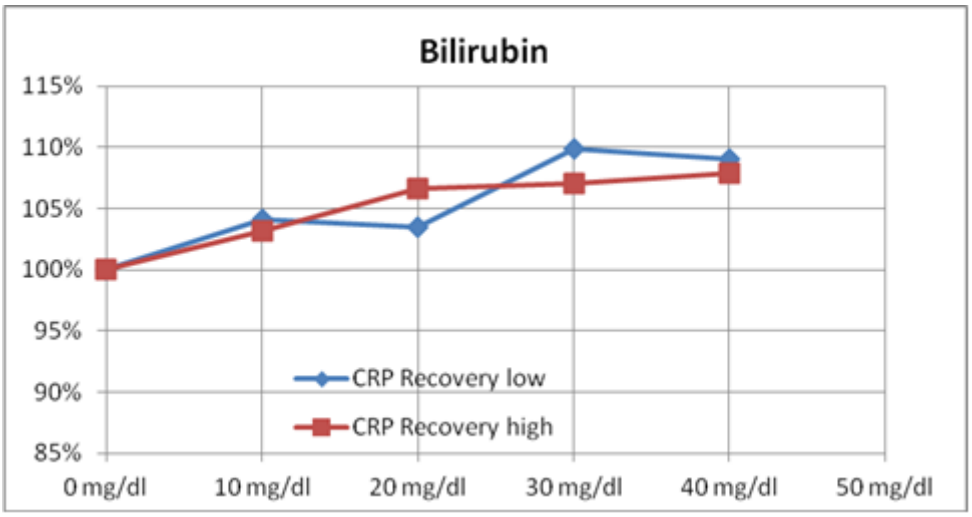

Figure 9 Bilirubin Interference

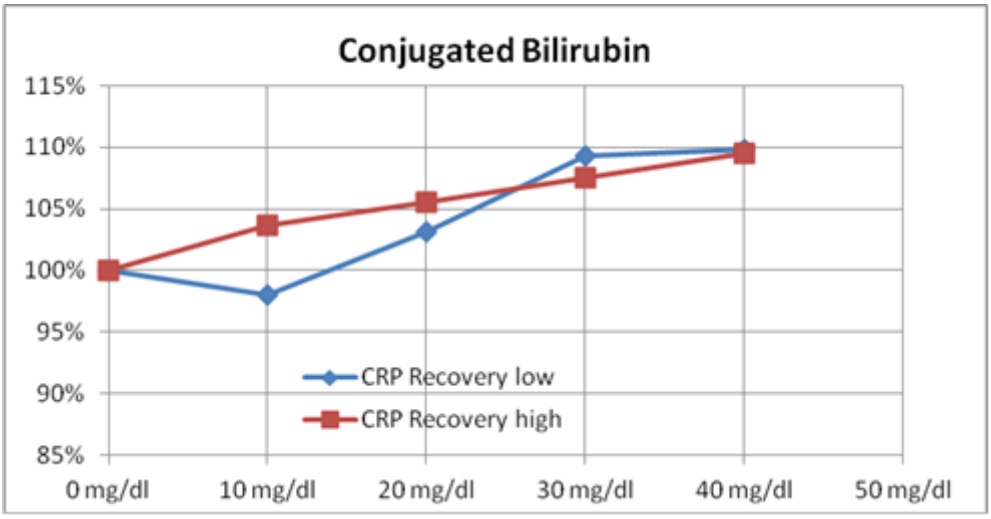

Figure 10 Conjugated Bilirubin Interference

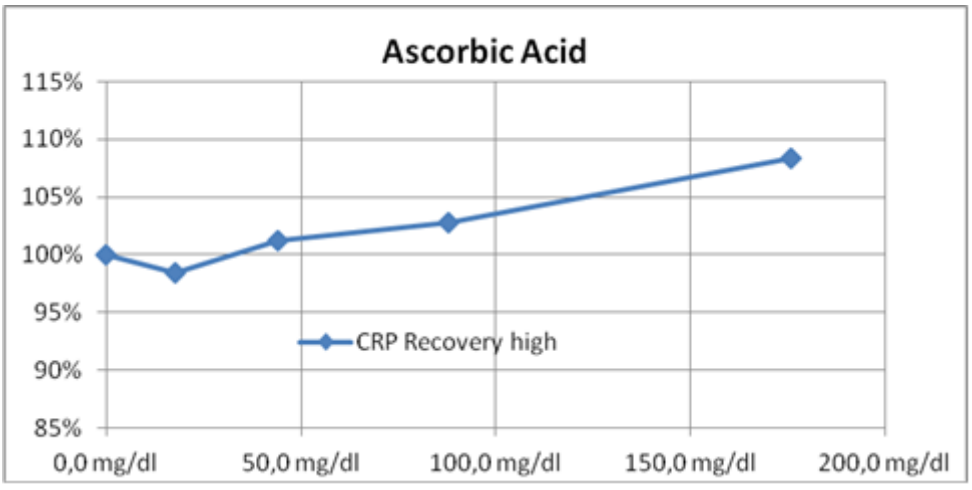

Figure 11 Ascorbin Interference

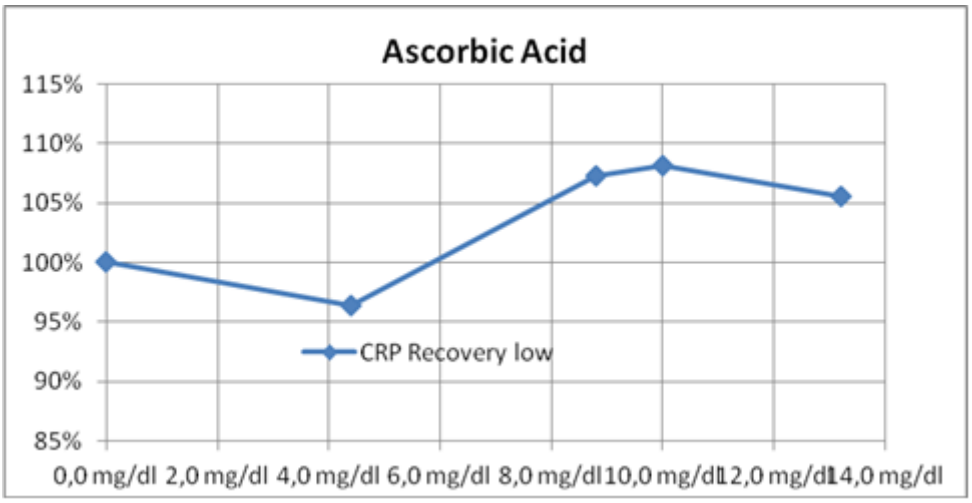

Figure 12 Ascorbic Acid Interference

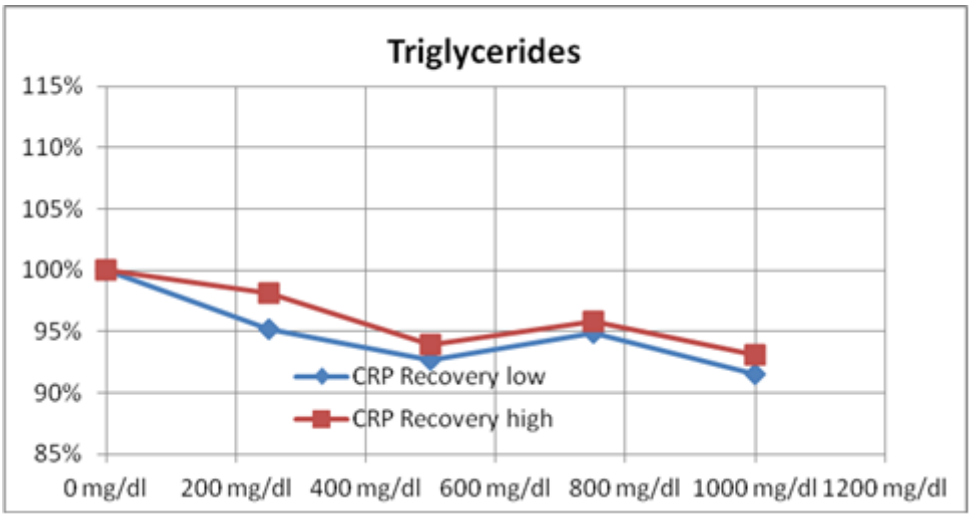

Figure 13 Triglycerides Interference

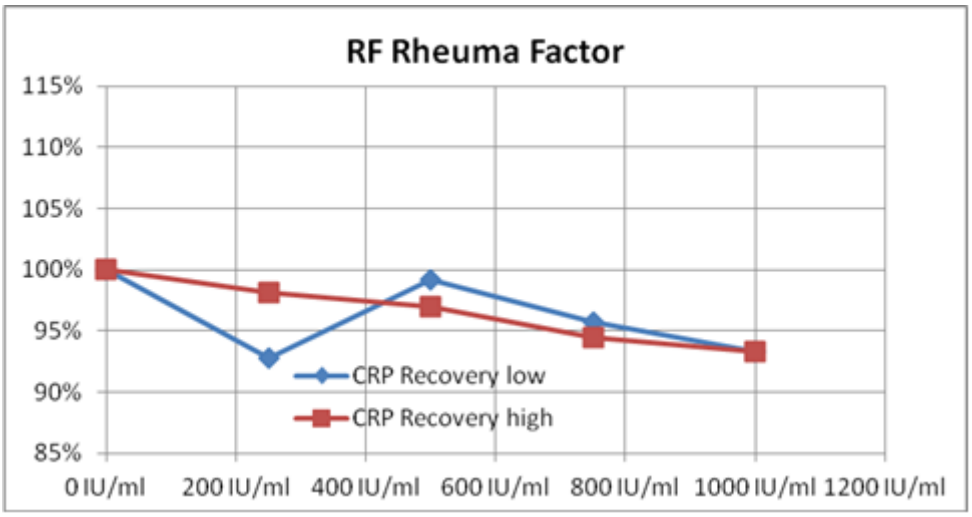

Figure 14 Rheuma Factor Interference

7. Traceability of CRP Calibration: External QC materials matrix and sample materials

The Eurolyser CRP assay was designed to be used with fresh whole blood (direct finger blood or EDTA samples) and serum.

Plasma (EDTA as well as Li-Heparin) was not in the scope of the development.

The buffer solution used within the Eurolyser CRP assay was designed to lyse the red blood cells and it acts as the main buffer solution wherein the binding with Antigen/Antibodies takes place.

This buffer solution causes a matrix effect on any other sample material which is not native serum or native whole blood.

Most Calibrators and external QC Controls are Plasma based.

Those calibrators and controls include preservatives as well as stabilizers. For this reason external QC material and calibration material (such as reference materials) are not directly compatible with the Eurolyser CRP assay.

External QC materials and Calibrators show a high positive bias. Patients Plasma samples show negative as well as positive bias (~ 10-20%) depending on the anticoagulant used (Li Hep EDTA Sodium Citrate etc.).

In order to guarantee the traceability to a high reference material all production Lots of CRP testkits are calibrated as follows:

50 fresh serum patient samples distributed over the linearity range of the assay are sourced at local hospitals.
Those 50 samples are measured on Abbott Architect ci8200 calibrated against **IFCC ERM DA474** or higer.

The 50 serum samples with the CRP concentrations measured with Abbott Architectci8200 are taken as the calibrator set for Eurolyser CRP.

By using those 50 samples Eurolyser establishes the master calibration curve.

Internal QC material is assigned by measuring against this master calibration curves and the values of those QC material is then assigned. The assay therefore is traceable to IFCC ERMDA474 (or higher) standards.
The whole blood calibration curve is set by using the mathematical equation of Serum versus whole blood signal strength in consideration of the Hematocrit influence of whole blood samples.

CRP Calibration Model