Evaluation of the SMART hsCRP testkit

Eurolyser Diagnostica GesmbH
Bayernstrasse 11A
5020 Salzburg

November, 2008

Author: Michael Gruber

Installation and orientation
The Eurolyser smart (single method automated reading technology) is intended to provide a precise, user-friendly measurement system for rapid, direct ascertainment of CRP / hsCRP concentrations from whole blood and serum, as a true point-of-care system.

The device provided for evaluation (Ser. No. Ab0647) is a production device that, within 0.5 hours, was set up, initialized, and configured as a stand-alone device, with no IT connection.

Scope and goals of the evaluation

1 Method comparison

Review of the conformance of the hsCRP measurement results from the SMART 700 from whole blood / serum, and the results from the clinical-chemistry fully automated EurolyserCCA180 from serum.

2. Imprecision

Characterization of the precision of the SMART 700 production model for various hsCRP concentrations.

3. Linearity

Determination of the linearity of the CRP measurement range of the SMART 700.

4. Interference:
Validation of Interference from typical interfering substances

5. Samples, reagents, and consumables

For the method comparison, daily routine laboratory samples that were no longer needed of (EDTA) whole blood, corresponding serum, were set aside, and measured on the same day.The sample have been collected at several hospitals near Salzburg.

Material and methods

SMART analyser

The SMART 700 with its attendant hsCRP test kit allows quantitative immunoturbidimetric tests in 2 concentration ranges:

Serum: 0.25-40 mg/L and whole blood 0,50-40 mg/l.

The ERS (Eurolyser Reagent System) cuvettes used are pre-filled with 1000 µL of Buffer Reagent Glycine Buffer 170 mM; the ERS caps that are placed on the cuvettes are pre-filled with 250 µL of Latex Reagent (rabbit anti-human –RP antibodies, 0.2%). The CRP concentration is calculated from the photometric ascertainment of the agglutination kinetics at 700 nm (or 546nm), compared to human CRP.

Reagent charges:

hsCRP kit LOT 878021

Control materials:
Low and high control “LOT 509214”

For each measurement with the Smart , 20 µL of a sample is pipetted into an ERS cuvette, the ERS cap is place on it, and the cuvette is placed into the device. The Smart analyser then measures whole blood samples, depending on the hematocrit value, which is determined in advance and input to the device, and serum and plasma samples without this information.

Eurolyser CCA180

The testing principle of the EurolyserCCA180 (C-Reactive Protein High Sensitive Assay) is based on a particle-amplified immunological turbidimetric test from Pointe Scientific USA, for which reagents R1, R2 are supplied in ready-to-use solution. The measurement range for the CCA180 hsCRP is 0.10 to 320 mg/L, according to the manufacturer’s data. Only the serum samples were used here.

Linearity

To determine the linearity, three measurements were carried out within the following linearity ranges, whereby the following dilution series were applied:

Control Serum (with bidistilled water):
Whole blood (with physiological saline solution):

Final CRP concentration	Serum sample volume	Bidistilled water volume
40 mg/L	Initial solution	
20 mg/L	100 µL of initial solution	100 µL
5 mg/L	100 µL of the 20 mg/L sol.	300 µL
1 mg/L	100 µL of the 5 mg/L sol.	400 µL
0,5 mg/L	100 µL of the 1 mg/L sol.	100 µL
0,25 mg/L	100 µL of the 0,5 mg/L sol.	100 µL

The dilution series for the whole blood sample was applied as follows:

Final CRP concentration	Whole blood sample volume	NaCl volume
33 mg/L	Initial solution	
8 mg/L	100 µL of initial solution	313 µL
2 mg/L	100 µL of the 8 mg/L sol.	300 µL
1 mg/L	100 µL of the 2 mg/L sol.	100 µL
0,5 mg/L	100 µL of the 1 mg/L sol.	100 µL
0,25 mg/L	100 µL of the 0,5 mg/L sol.	100 µL

The dilution series for the serum samples was applied as follows:

5. Analysis

The CRP results were documented with appropriate peripheral devices or, for the SMART , by manual entry and transformed into an EXCEL file for statistical analysis.

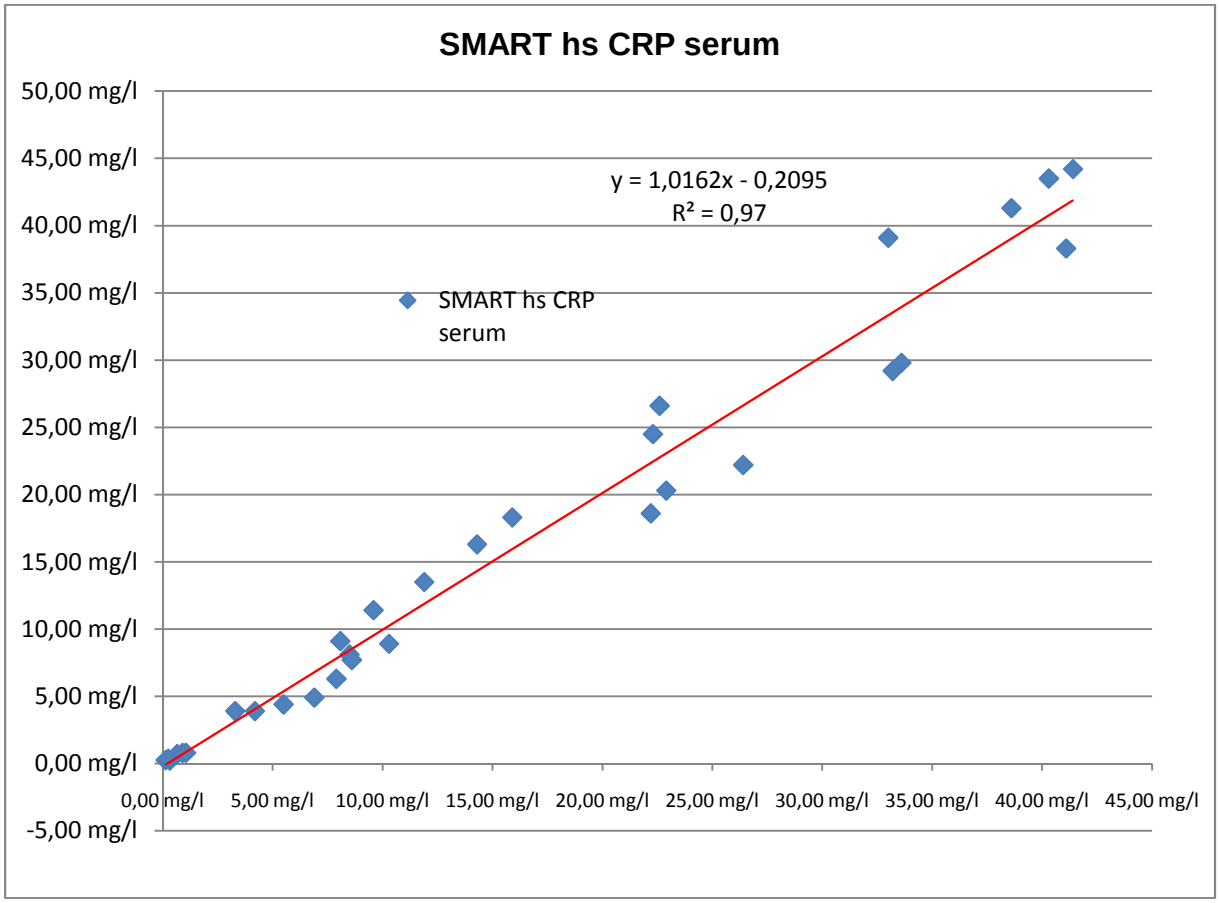
6.1 Method comparison

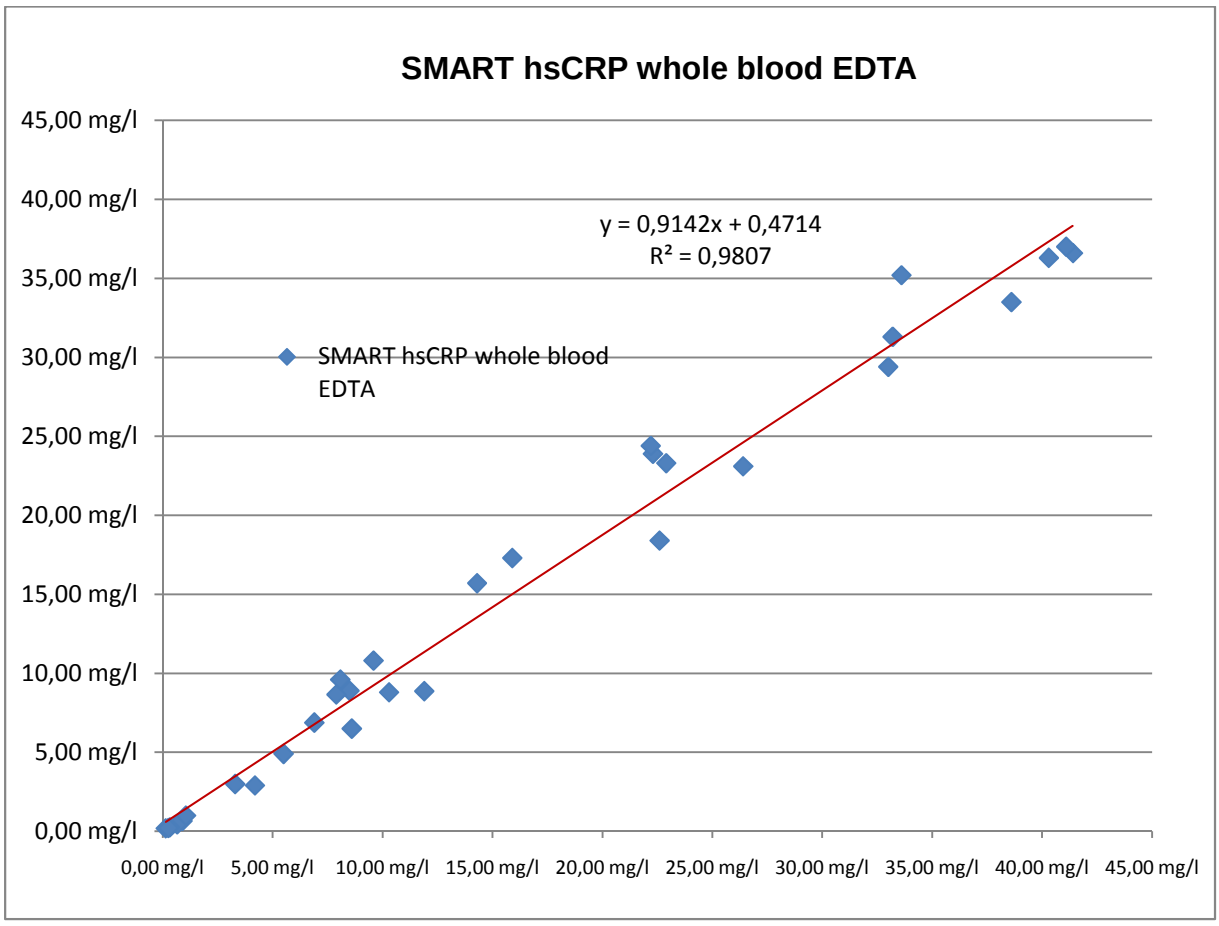
The method comparison was carried out with 31 samples each of EDTA-whole blood, and serum, over a range of concentration from 0.25 – 40 mg/L in the SMART , and compared to the serum results from the EurolyserCCA180.

The raw data are shown in the following table:

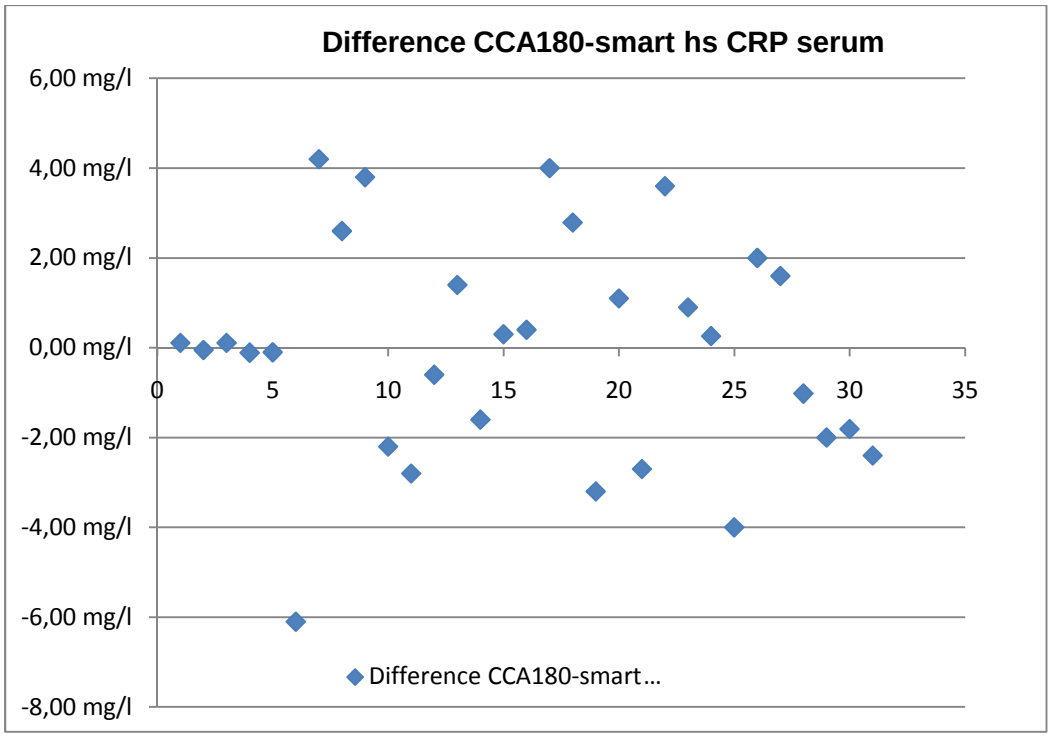
Patient No	CCA180 hsCRP (reference)	SMART hs CRP serum	HCT (%)	SMART hsCRP whole blood EDTA
1	0,90 mg/l	0,79 mg/l	51%	0,66 mg/l
2	0,66 mg/l	0,71 mg/l	44%	0,44 mg/l
3	0,33 mg/l	0,22 mg/l	43%	0,25 mg/l
4	0,14 mg/l	0,25 mg/l	38%	0,19 mg/l
5	0,25 mg/l	0,35 mg/l	48%	0,21 mg/l
6	33,00 mg/l	39,10 mg/l	51%	29,40 mg/l
7	26,40 mg/l	22,20 mg/l	55%	23,10 mg/l
8	22,90 mg/l	20,30 mg/l	44%	23,30 mg/l
9	33,60 mg/l	29,80 mg/l	39%	35,20 mg/l
10	22,30 mg/l	24,50 mg/l	44%	23,90 mg/l
11	41,40 mg/l	44,20 mg/l	37%	36,60 mg/l
12	3,30 mg/l	3,90 mg/l	40%	2,99 mg/l
13	10,30 mg/l	8,90 mg/l	41%	8,80 mg/l
14	11,90 mg/l	13,50 mg/l	43%	8,87 mg/l
15	4,20 mg/l	3,90 mg/l	46%	2,90 mg/l
16	8,50 mg/l	8,10 mg/l	48%	8,90 mg/l
17	33,20 mg/l	29,20 mg/l	39%	31,30 mg/l
18	41,09 mg/l	38,30 mg/l	40%	37,00 mg/l
19	40,30 mg/l	43,50 mg/l	43%	36,30 mg/l
20	5,50 mg/l	4,40 mg/l	44%	4,90 mg/l
21	38,60 mg/l	41,30 mg/l	49%	33,50 mg/l
22	22,20 mg/l	18,60 mg/l	53%	24,40 mg/l
23	8,60 mg/l	7,70 mg/l	53%	6,50 mg/l
24	1,06 mg/l	0,80 mg/l	52%	0,99 mg/l
25	22,60 mg/l	26,60 mg/l	44%	18,40 mg/l
26	6,90 mg/l	4,90 mg/l	38%	6,88 mg/l
27	7,90 mg/l	6,30 mg/l	36%	8,66 mg/l
28	8,08 mg/l	9,10 mg/l	33%	9,60 mg/l
29	14,30 mg/l	16,30 mg/l	39%	15,70 mg/l
30	9,59 mg/l	11,40 mg/l	30%	10,80 mg/l
31	15,90 mg/l	18,30 mg/l	31%	17,30 mg/l

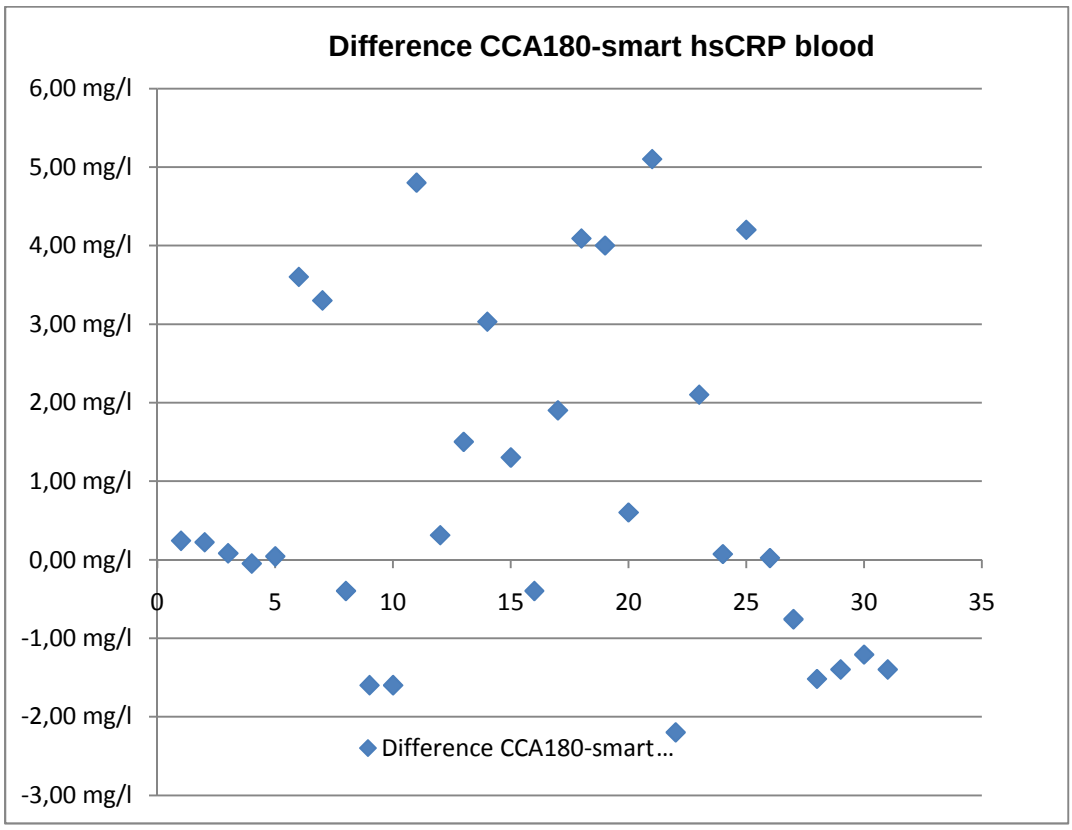
Table Method Comparisson


SMART hs CRP serum
50,00 mg/l
45,00 mg/l
40,00 mg/l
35,00 mg/l
30,00 mg/l
25,00 mg/l
20,00 mg/l
15,00 mg/l
10,00 mg/l
5,00 mg/l
0,00 mg/l
-5,00 mg/l
y = 1,0162x - 0,2095
R² = 0,97
SMART hs CRP serum
0,00 mg/l
5,00 mg/l
10,00 mg/l
15,00 mg/l
20,00 mg/l
25,00 mg/l
30,00 mg/l
35,00 mg/l
40,00 mg/l
45,00 mg/l


SMART hsCRP whole blood EDTA
45,00 mg/l
40,00 mg/l
35,00 mg/l
30,00 mg/l
25,00 mg/l
20,00 mg/l
15,00 mg/l
10,00 mg/l
5,00 mg/l
0,00 mg/l
y = 0,9142x + 0,4714
R² = 0,9807
SMART hsCRP whole blood EDTA
0,00 mg/l
5,00 mg/l
10,00 mg/l
15,00 mg/l
20,00 mg/l
25,00 mg/l
30,00 mg/l
35,00 mg/l
40,00 mg/l
45,00 mg/l

Difference Blots:





6.2 Imprecision:

The imprecision of the hsCRP measurement with the SMART 700 was determined in a series of 20 measurements in the range of the following concentrations:

Serum: ~5mg/L, 20mg/L,

Whole blood: ~5mg/L, ~20mg/L,

The standard deviations were according to

$S = (\sum (X - X_i)^2/(n-1))^{1/2}$

Where x = mean, x_i = measured value, and n = number of samples. The relative standard deviation S_{rel} was calculated according to: $S_{rel} = (S/ X) * 100\%$ Srel=cv%

The CRP values for the imprecision measurement of serum / and whole blood samples with the SMART 700 are summarized in the following Table

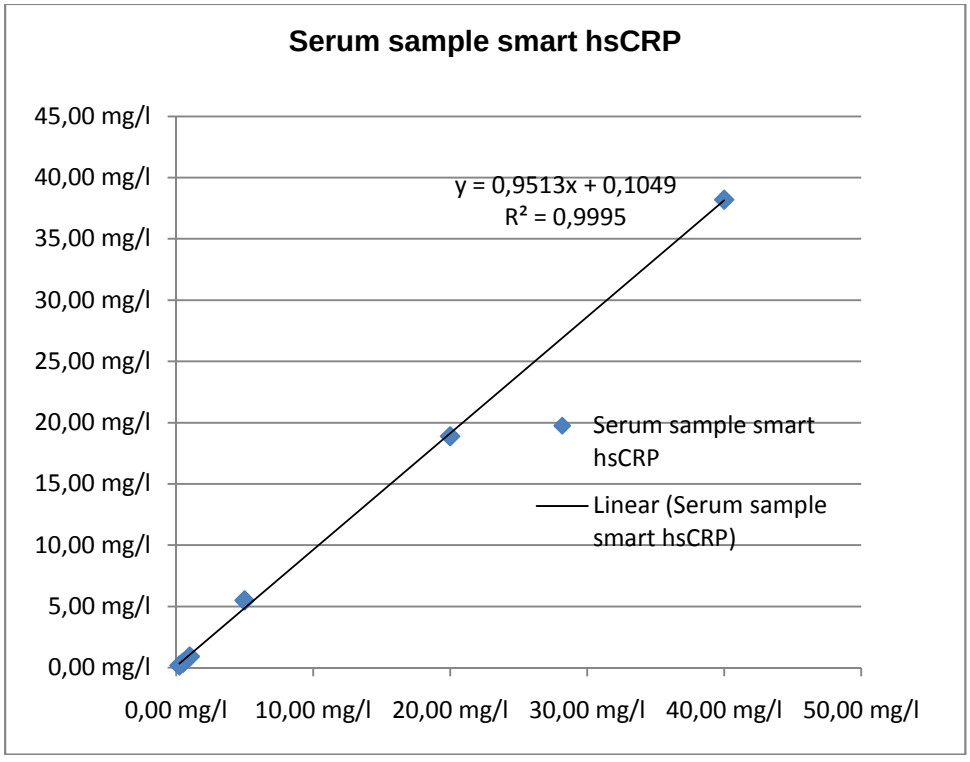
	serum low 5mg/l	**serum high 20mg/l**	**whole blood low 5mg/l**	**whole blood high 20 mg/l**
1	4,4 mg/l	20,10 mg/l	4,3 mg/l	20,30 mg/l
2	5,1 mg/l	19,90 mg/l	5,4 mg/l	19,30 mg/l
3	4,4 mg/l	19,40 mg/l	4,1 mg/l	19,20 mg/l
4	5,3 mg/l	20,10 mg/l	5,4 mg/l	19,90 mg/l
5	4,7 mg/l	19,90 mg/l	4,3 mg/l	21,30 mg/l
6	4,7 mg/l	19,40 mg/l	4,4 mg/l	21,00 mg/l
7	4,9 mg/l	19,80 mg/l	5,5 mg/l	20,80 mg/l
8	5,1 mg/l	19,60 mg/l	5,1 mg/l	20,30 mg/l
9	4,8 mg/l	20,20 mg/l	4,5 mg/l	19,80 mg/l
10	4,8 mg/l	19,30 mg/l	5,4 mg/l	21,20 mg/l
11	5,1 mg/l	20,90 mg/l	5,0 mg/l	19,30 mg/l
12	4,5 mg/l	21,30 mg/l	5,1 mg/l	19,80 mg/l
13	4,8 mg/l	18,50 mg/l	4,8 mg/l	21,10 mg/l
14	4,6 mg/l	20,10 mg/l	4,7 mg/l	19,20 mg/l
15	4,5 mg/l	20,00 mg/l	4,9 mg/l	19,10 mg/l
16	5,0 mg/l	19,80 mg/l	5,0 mg/l	21,90 mg/l
17	4,7 mg/l	19,00 mg/l	4,7 mg/l	22,10 mg/l
18	4,8 mg/l	18,80 mg/l	4,8 mg/l	22,30 mg/l
19	4,6 mg/l	20,90 mg/l	4,6 mg/l	20,90 mg/l
20	4,9 mg/l	20,00 mg/l	4,8 mg/l	20,00 mg/l
mean	4,79	19,85	4,84	20,44
Stabwn	0,25	0,69	0,40	1,01
Cv	**5,22%**	**3,48%**	**8,36%**	**4,96%**

6.2 Linearity

The following Table shows the results of the linearity measurement for serum.

Theoretical Final hsCRP concentration	Serum sample smart hsCRP
40,00 mg/l	38,20 mg/l
20,00 mg/l	18,90 mg/l
5,00 mg/l	5,50 mg/l
1,00 mg/l	0,92 mg/l
0,50 mg/l	0,41 mg/l
0,25 mg/l	0,20 mg/l

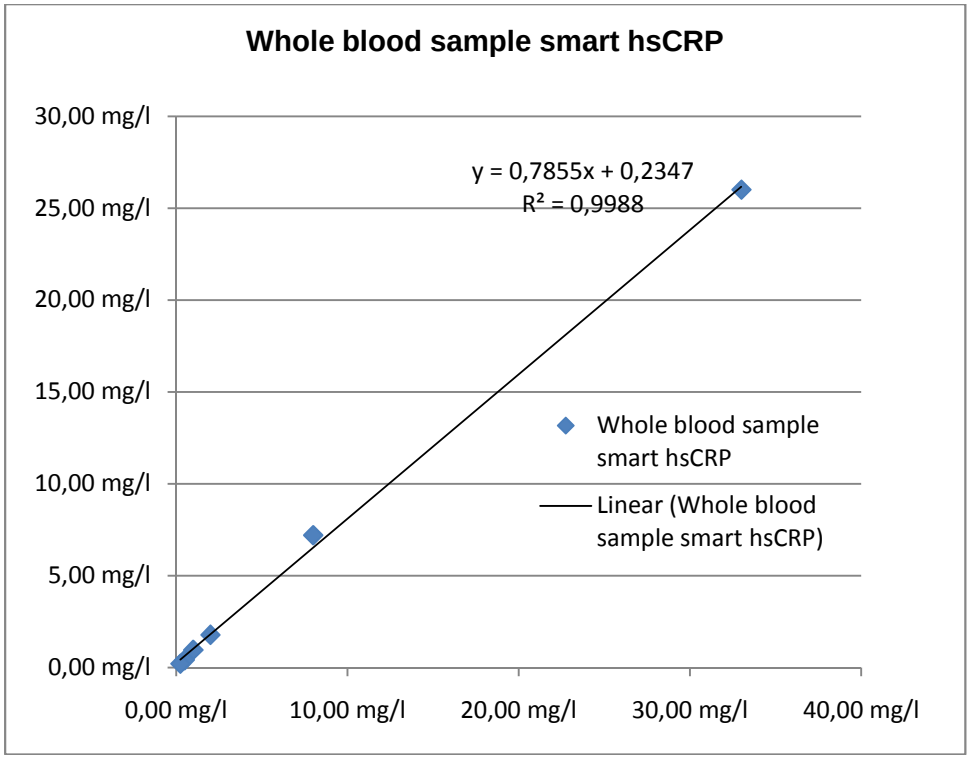
If the hsCRP concentrations of the diluted serum samples actually measured with the SMART700 are plotted against the theoretical calculations , then a very good correlation of r=0.995, within the pipetting error, is found.



Linearity of hsCRP ascertainment with the SMART 700 with whole blood

theoretical Final hsCRP concentration blood	Whole blood sample smart hsCRP
33,00 mg/l	26,00 mg/l
8,00 mg/l	7,20 mg/l
2,00 mg/l	1,78 mg/l
1,00 mg/l	0,96 mg/l
0,50 mg/l	0,41 mg/l
0,25 mg/l	0,21 mg/l

If the hsCRP concentrations of the diluted whole blood samples actually measured with the SMART 700 are plotted against the theoretical calculations then a very good correlation of r=0.998, within the pipetting error, is found here as well.

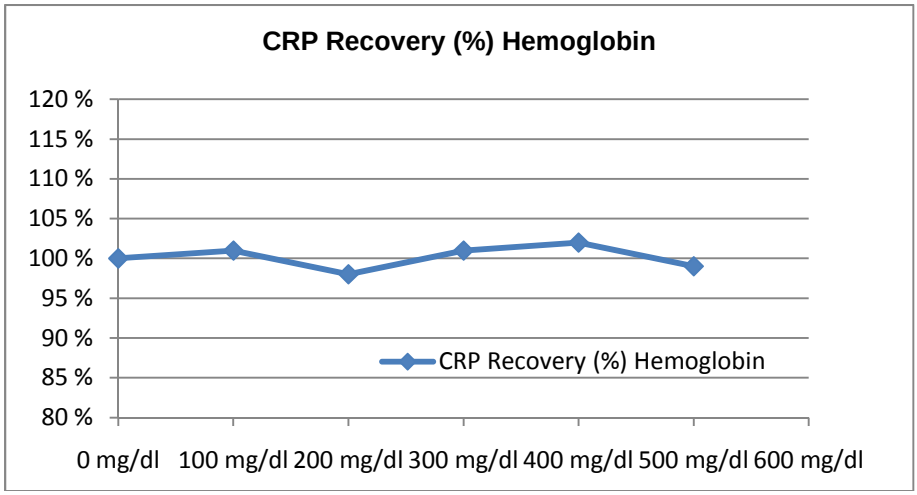


6.3 Interference:

Interference has been tested with spiked material for Hemoglobin,Bilirubin,Triglycerides and been found as non significant in terms of recovery of results

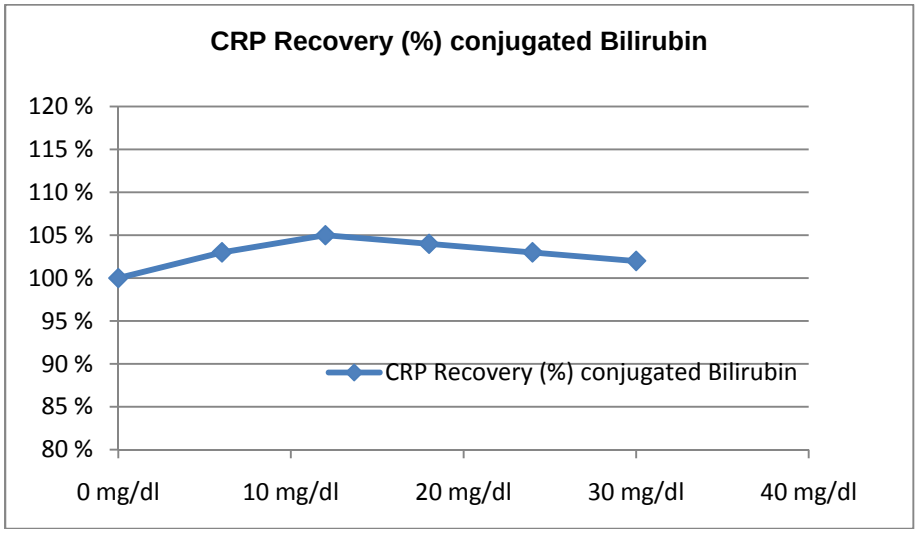
Hemoglobin:

Hemoglobin	CRP Recovery (%) Hemoglobin
0 mg/dl	100 %
100 mg/dl	101 %
200 mg/dl	98 %
300 mg/dl	101 %
400 mg/dl	102 %
500 mg/dl	99 %



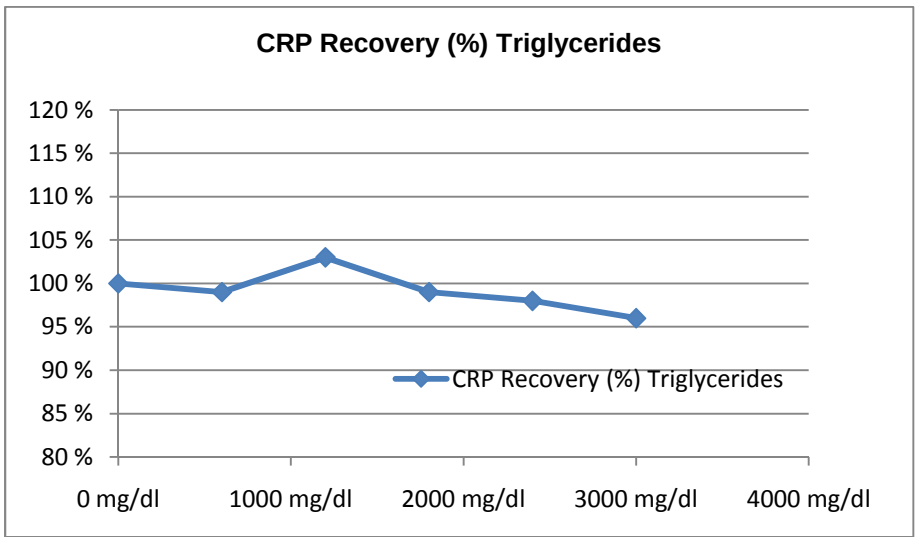
Bilirubin:

conjugated Bilirubin	CRP Recovery (%) conjugated Bilirubin
0 mg/dl	100 %
6 mg/dl	103 %
12 mg/dl	105 %
18 mg/dl	104 %
24 mg/dl	103 %
30 mg/dl	102 %



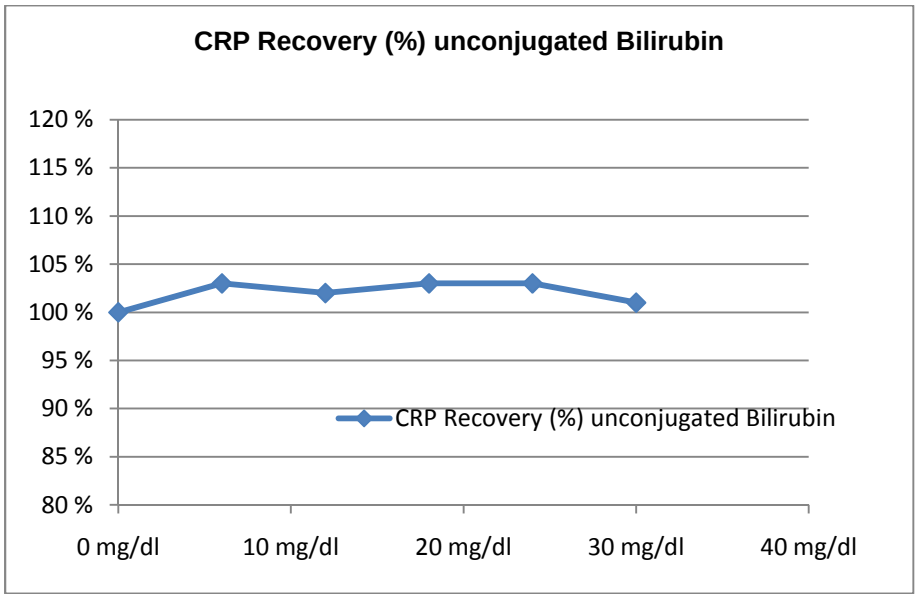
Triglycerides:

Triglycerides	CRP Recovery (%) Triglycerides
0 mg/dl	100 %
600 mg/dl	99 %
1200 mg/dl	103 %
1800 mg/dl	99 %
2400 mg/dl	98 %
3000 mg/dl	96 %



Unconjugated Bilirubin

unconjugated Bilirubin	CRP Recovery (%) unconjugated Bilirubin
0 mg/dl	100 %
6 mg/dl	103 %
12 mg/dl	102 %
18 mg/dl	103 %
24 mg/dl	103 %
30 mg/dl	101 %



Summary

The SMART hsCRP is designed for locally required laboratory diagnostics, to provide quantitative hsCRP values from whole blood as well as from serum , rapidly and with high validity. Its use is easy to learn, operation is simple and robust enough that even personnel outside of the laboratory can work with the device and the required monitoring and quality measures after an appropriate period of orientation.

The SMART700 measurement device, tested against the CCA180 measurement method as a reference, showed very good correlation between measurements in whole blood mode and the serum reference.

The SMART 700 can be considered an excellent POCT measurement device for ascertainment from whole blood and serum.

The precision of the SMART 700 is, as expected, higher for hsCRP ascertainment from serum samples than from whole blood samples; however, it appears to be absolutely suitable for practical purposes here, as well.

The linearity of the tested device / reagent combination is also considered to be very good.

In summary, it can be determined that the SMART 700 has excellent practical applicability for the concepted application, and good measurement characteristics, especially for measurements with whole blood samples.

Michael Gruber
Salzburg, Nov.2008