Evaluation of the SMART 546 CRP measurement system

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SMART 546 Evaluation Report Freiburg

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1. Description of SMART 546 system configuration

2. Installation and orientation

The Eurolyser smart (single method automated reading technology 546 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. Aa0166) is a production device that, within 0.5 hours, was set up, initialized, and configured as a stand-alone device, with no IT connection. Training of the personnel (2 med-tech lab assistants) took 1.0 hours, and was provided by manufacturer's organizing team (Peter Himmelsbach, Sysmex).

3. Scope and goals of the evaluation

1 Method comparison

Review of the conformance of the CRP/hsCRP measurement results from the SMART 546 from whole blood / EDTA plasma / serum, and the results from the clinical-chemical fully automated Cobas 6000 (Roche Diagnostics, Mannheim) from serum.

2. Imprecision

Characterization of the precision of the SMART 546 production model for various CRP concentrations.

3. Linearity

Determination of the linearity of the CRP measurement range of the SMART 546.

4. Samples, reagents, and consumables

Samples

For the method comparison, daily routine laboratory samples that were no longer needed of (EDTA) whole blood, corresponding serum, and, after centrifuging of the whole blood sample, EDTA plasma aliquot were set aside, and measured on the same day.

Material and methods

SMART 546

The SMART 546 with its attendant CRP test kit allows quantitative immunoturbidimetric tests in 2 concentration ranges:

Serum/Plasma: 0.5-120 mg/L for hs - CRP
and
whole blood: 2-200 mg/L for CRP.

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 200 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 546 nm, compared to human CRP.

Reagent charges:

see table, "Provision and use of reagents," Appendix II.

The repeat measurements for the method comparison were carried out with Charge No. 0629901 (2008-03).

Control materials:

Low and high control "CAL523/02" (09.2007) / Eurolyser Diagnostica GmbH

Procedure:

For each measurement with the Smart 546, 5 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 546 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.

Cobas 6000

The testing principle of the Cobas 6000 CRPHS (C-Reactive Protein High Sensitive Assay) is based on a particle-amplified immunological turbidimetric test, for which reagents R1, R2 are supplied in ready-to-use solution. The measurement range for the Cobas 6000 CRPHS is 0.15 to 300 mg/L, according to the manufacturer's data. ONLY THE serum samples were used here.

Reagent charges:

682354 (10/2008)

Control materials:

CRP-T-Control N: Charge No. 17818802 (01/2010) and Precipath Protein: Charge No. 17833 (04/2009), (Roche Diagnostics).

Procedure:

For the measurement with the Cobas 6000, the samples were placed in the rack intake, and the measurements were requested via software.

Linearity

To determine the linearity, three measurements were carried out within the following linearity ranges, whereby the following dilution series were applied:
Serum (with bidistilled water): >120mg/L, ~100mg/L, ~50mg/L, ~20mg/L, ~5mg/L, ~2mg/L, ~0.5mg/L Whole blood (with physiological saline solution): >200mg/L, ~50mg/L, ~20mg/L, ~5mg/L, ~2mg/L

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

Final CRP concentration	Serum sample volume	Bidistilled water volume
120 mg/L	Initial solution	
100 mg/L	50 µL of initial solution	10 µL
50 mg/L	40 µL of the 100 mg/L sol.	40 µL
20 mg/L	50 µL of the 50 mg/L sol.	75 µL
5 mg/L	50 µL of the 20 mg/L sol.	150 µL
2 mg/L	20 µL of the 5 mg/L sol.	30 µL
0,5 mg/L	10 µL of the 2 mg/L sol.	30 µL

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

Final CRP concentration	Whole blood sample volume	NaCl volume
170 mg/L	Initial solution	
107 mg/L	170 µL of initial solution	100 µL
52 mg/L	100 µL of the 107 mg/L sol.	105 µL
20 mg/L	50 µL of the 52 mg/L sol.	80 µL
5 mg/L	20 µL of the 20 mg/L sol.	60 µL

2 mg/L	20 µL of the 5 mg/L sol.	30 µL

5. Analysis

The CRP results were documented with appropriate peripheral devices (Cobas 6000: system printer) or, for the SMART 546, by manual entry by the med tech assistant, and transformed into an EXCEL file for statistical analysis (dual control).

6. Results

The setup, commissioning, and orientation in the evaluation laboratory were speedy, smooth, and very effective. After 0.5 hours, a med tech lab assistant is able to determine CRP levels with the SMART 546.

6.1 Method comparison

The method comparison was carried out with 50 samples each of EDTA-whole blood, plasma, and serum, over a range of concentration from 0.2 – 120 mg/mL in the SMART 546, and compared to the serum results from the Cobas 6000 in accordance with Passing-Bablock (1). The raw data are listed in the appendix. Fig. 1 shows the correlation between the CRP ascertainment from serum with the SMART 546 and Cobas 6000.

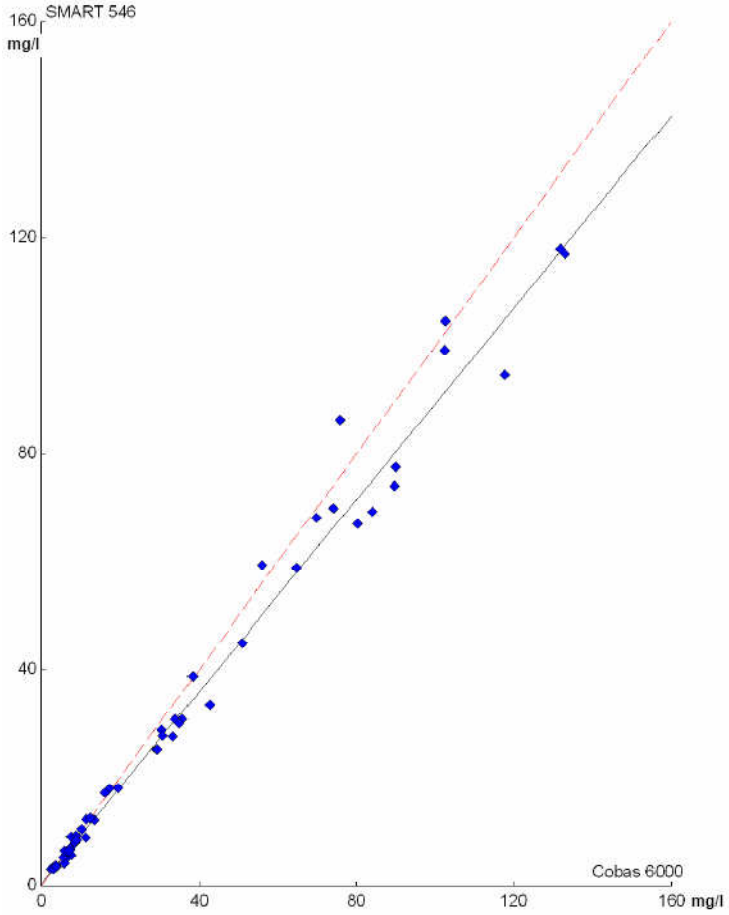

Fig. 1: Comparison of COBAS 6000 (reference) and SMART 546 Serum r=0.991
Passing-Bablok agreement test N = 50
Slope: 0.889 [0.858 to 0.933]
Intercept: 0.38 [-0.07 to 0.79]

Figure 2 shows the difference plot between the reference values (Cobas; mean) and the corresponding SMART 546 serum comparison values.

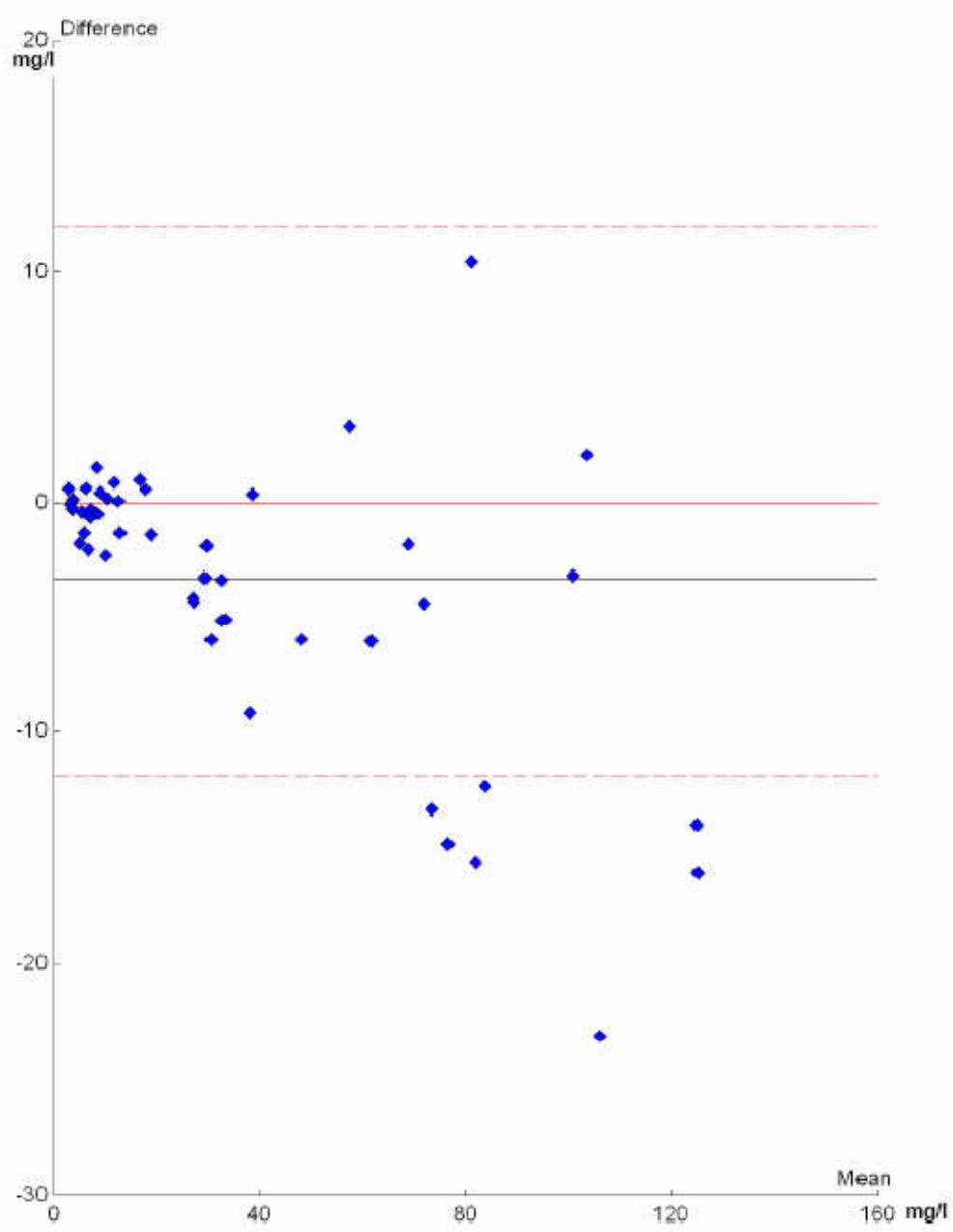

Fig. 2: Comparison COBAS 6000 (reference) and SMART 546 Serum r = 0,991
Difference plot N = 50 Mean
Mean difference: -3.29 [-4.98 to -1.59]

Fig. 3 shows the correlation between the SMART 546 CRP plasma results and the Cobas 600 serum reference.

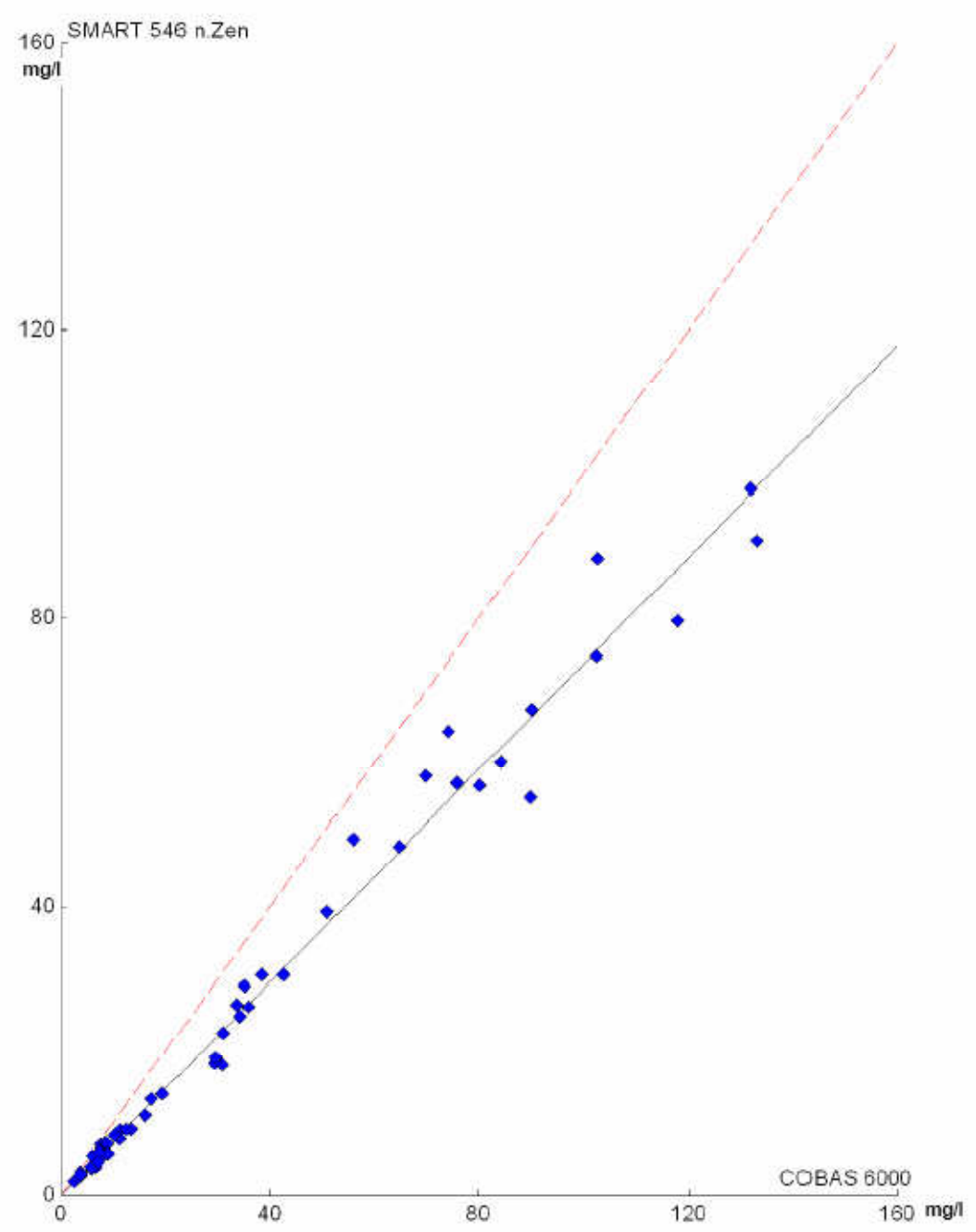

Fig. 3: Comparison of COBAS 6000 (reference) and SMART 546 plasma r=0.991
Passing-Bablok agreement test N = 50
Slope : 0.737 [0.711 to 0.757]
Intercept: 0.01 [-0.52 to 0.40]

Figure 4 shows the difference plot between the reference values (Cobas; mean) and the corresponding SMART 546 plasma comparison values.

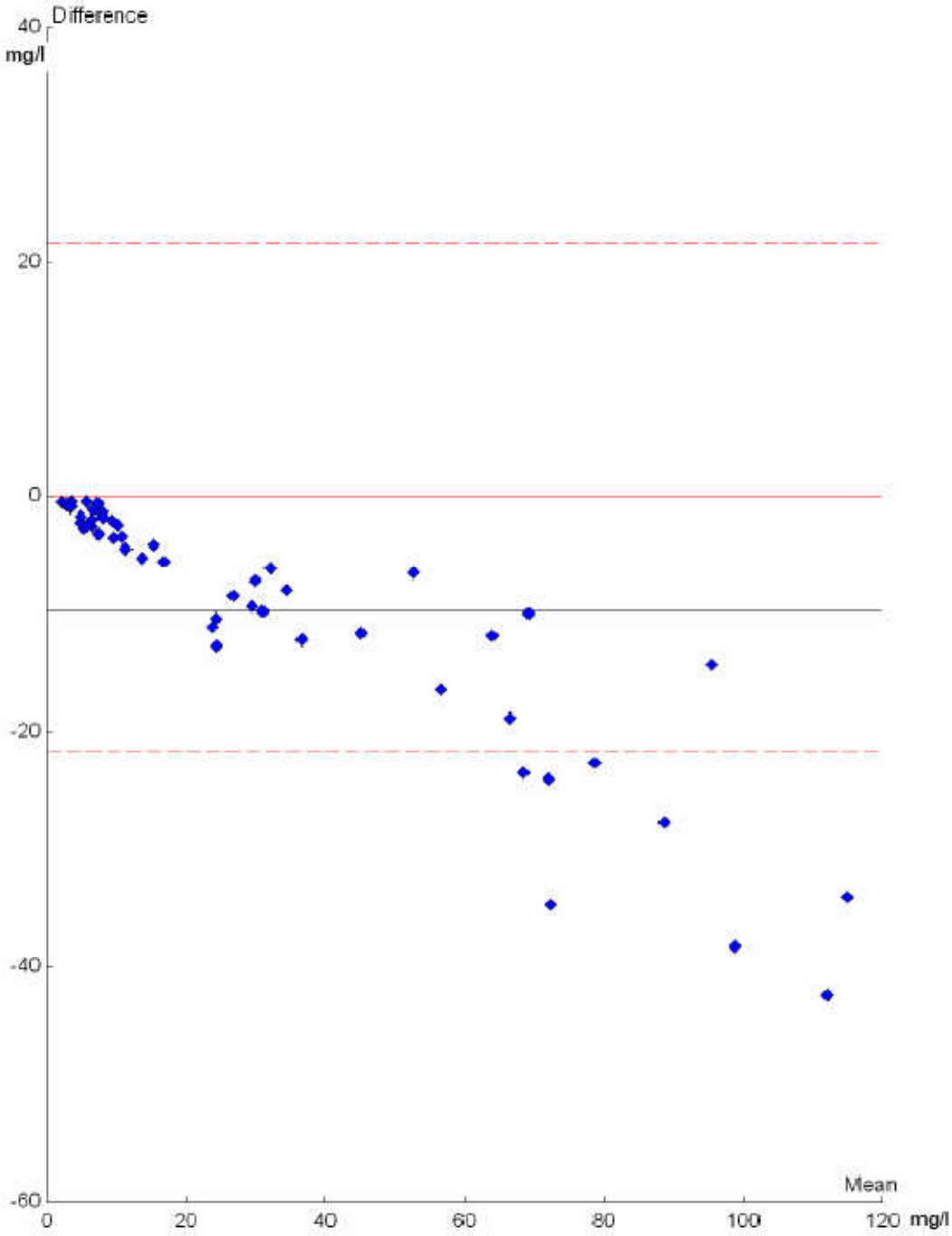

Fig. 4: Comparison of COBAS 6000 (reference) and SMART 546 plasma r=0.991
Difference plot N = 50
Mean difference: -9.76 [-12.8 to -6.71]

Fig. 5 shows the correlation between the SMART 546 CRP whole blood results and the Cobas 600 serum reference.

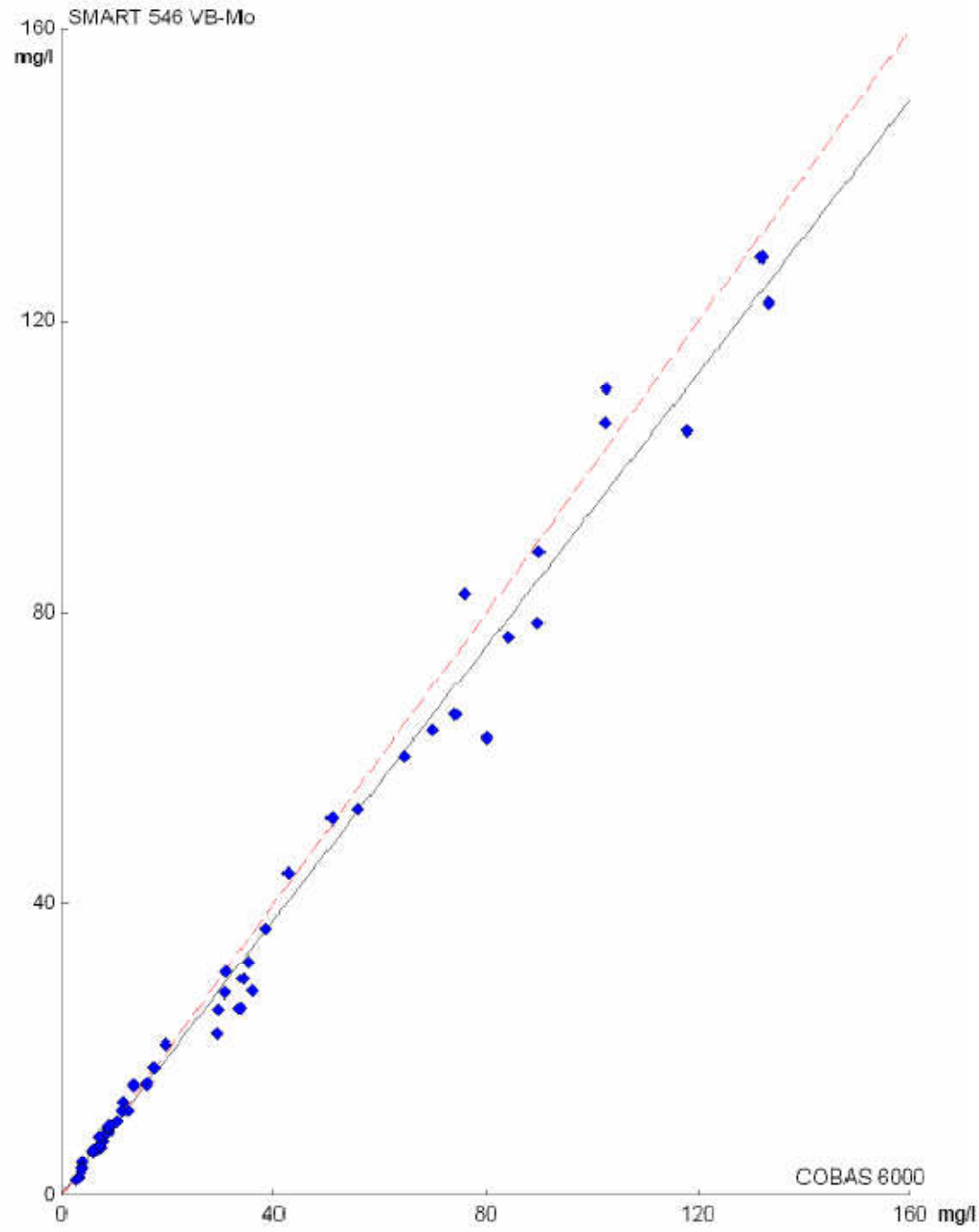

Fig. 5: Passing-Bablok agreement test N = 5
Slope : 0.938 [0.906 to 0.981]
Intercept: 0.32 [-0.15 to 0.82]

Figure 6 shows the difference plot between the reference values (Cobas; mean) and the corresponding SMART 546 whole blood comparison values.

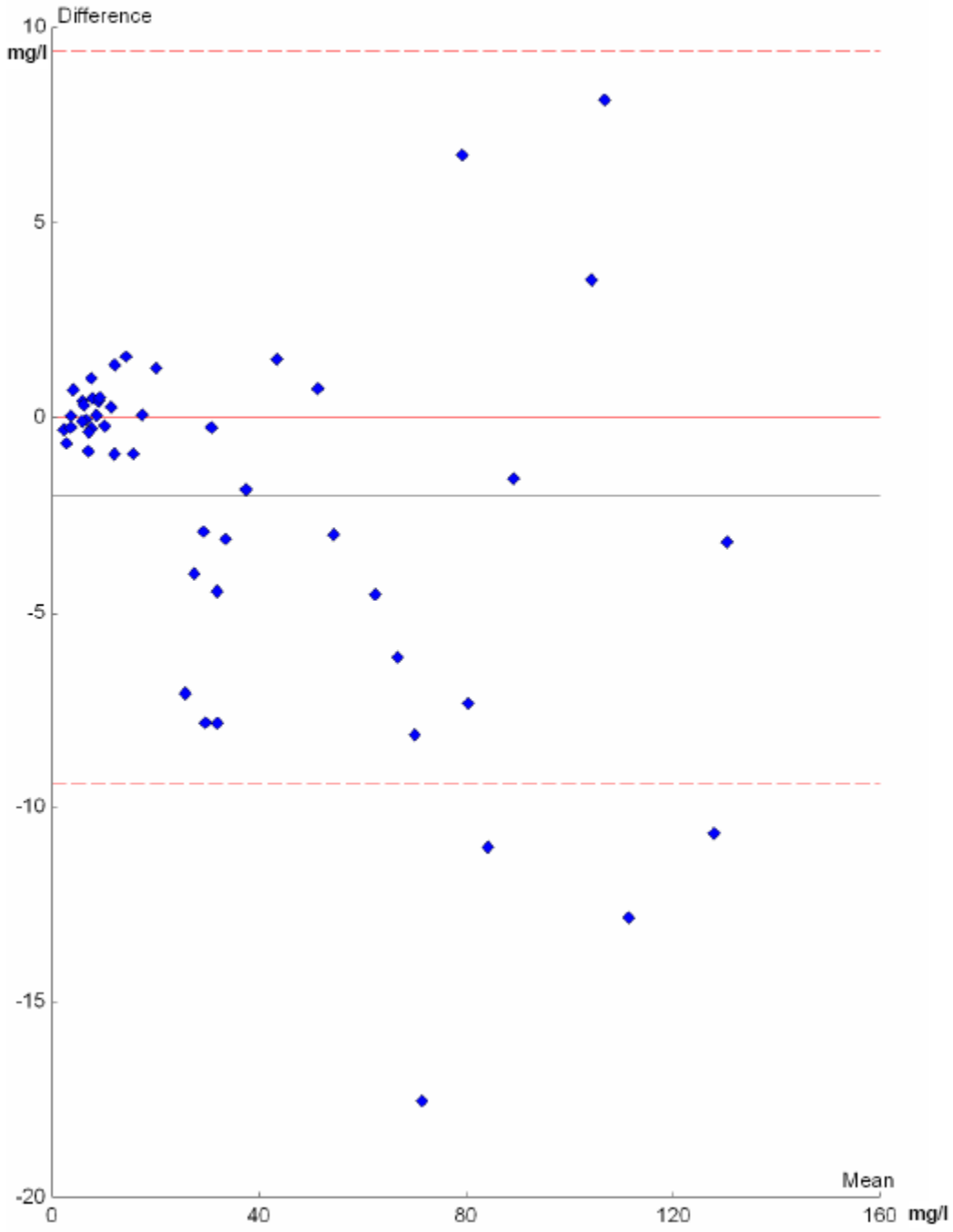

Fig. 6: Comparison of COBAS 6000 (reference) and SMART 546 whole blood r=0.993
Difference plot N = 50
Mean difference: -2.02 [-3.35 to -0.683]

6.2 Imprecision

The imprecision of the CRP measurement with the SMART 546 was determined in a series of 20 measurements in the range of the following concentrations:
Serum: ~1mg/L, ~5mg/L, ~20mg/L, ~100mg/Ll
Whole blood: ~5mg/L, ~20mg/L, ~150-200mg/L
The standard deviations were according to
$S = (\sum (X_m - X_i)^2/(n-1))^{1/2}$
Where x_m = mean, x_i = measured value, and n = number of samples. The relative standard deviation S_{rel} was calculated according to: $S_{rel} = (S/ X_m) * 100\%$

The CRP values for the imprecision measurement of serum / plasma and whole blood samples with the SMART 546 are summarized in Table 1.

Table 1: Imprecision of CRP ascertainment in three concentration ranges and two matrices with the SMART 546

CRP-Konz.	Serum/Plasma				Vollblut		
Datum:	18.06.2007	18.06.2007	18.06.2007	19.06.2007	19.06.2007	19.06.2007	*WH 26.06.07
Bereich	~1mg/L	~5mg/L	~20mg/L	~100mg/L	~5mg/L	~20mg/L	~100-160mg/L
Nr.							
1	1,3	4,2	22,2	106,3	5,0	18,7	124,0
2	1,5	4,1	20,8	98,3	3,8	16,5	134,3
3	1,1	4,4	21,4	104,8	4,8	15,2	128,5
4	1,5	4,2	22,7	105,2	4,5	14,4	136,6
5	1,7	4,6	21,4	103,6	3,8	15,5	125,4
6	1,1	4,5	23,5	96,8	3,8	15,1	129,7
7	1,3	4,5	22,0	101,5	4,6	17,0	137,6
8	1,5	4,2	21,6	105,6	4,5	15,9	141,3
9	1,2	4,0	22,1	97,1	4,1	15,6	134,3
10	1,1	4,5	21,2	109,5	4,4	15,1	137,8
11	1,3	4,6	20,2	101,7	5,1	14,8	135,0
12	1,4	3,9	22,2	104,0	5,0	18,7	138,0
13	1,7	4,5	22,7	99,5	3,7	15,9	120,8
14	1,5	4,5	21,4	102,9	3,8	14,8	137,0
15	1,4	4,2	19,2	105,9	4,2	18,1	143,3
16	1,5	4,4	23,5	97,7	4,4	14,4	137,8
17	1,3	4,6	23,0	101,7	3,7	14,5	135,8
18	1,5	4,1	20,6	98,0	4,4	15,6	143,5
19	1,9	4,1	19,6	100,5	4,5	17,6	148,3
20	1,7	4,5	21,0	97,3	4,6	15,5	128,8
MW (x) [mg/mL]	1,4	4,3	21,6	101,9	4,3	15,9	134,9
S [mg/mL]	0,2	0,2	1,2	3,6	0,4	1,3	6,8
S_{rel} [%]	15,0	5,0	5,3	3,5	10,2	8,4	5,0

*WH = Wiederholung mit Test-Kits LOT-Nr. 629901-02

Appendix II: SMART 546 reagents used

6.3 Linearity

The following Table 2 shows the results of the linearity measurement for serum.

Table 2: Linearity of CRP ascertainment with the SMART 546 with serum

Konzentration CRP in mg/L	1.Wert	2.Wert	3.Wert
~120mg/L	114,3	120	117,5
~100mg/L	86,5	86,3	84,3
~50mg/L	54,2	53,8	56,2
~20mg/L	19,3	19,3	21,4
~5mg/L	4,7	3,7	3,4
~2mg/L	1,9	1,5	1,7
~0,5mg/L	0,4	0,4	0,4

If the CRP concentrations of the diluted serum samples actually measured with the SMART 546 are plotted against the theoretical calculations (Fig. 7), then a very good correlation of r=0.998, within the pipetting error, is found.

Table 3: Linearity of CRP ascertainment with the SMART 546 with whole blood

Konzentration CRP in mg/L	1.Wert	2.Wert	3.Wert
~170mg/L	165,6	173,8	171,2
~107mg/L	105,5	108,3	105,6
~52mg/L	54,2	59,6	57,2
~20mg/L	19,7	23,5	23,8
~5mg/L	6,6	7,5	6,5
~2mg/L	2,5	3	3

If the CRP concentrations of the diluted whole blood samples actually measured with the SMART 546 are plotted against the theoretical calculations (Fig. 8), then a very good correlation of r=0.999, within the pipetting error, is found here as well.

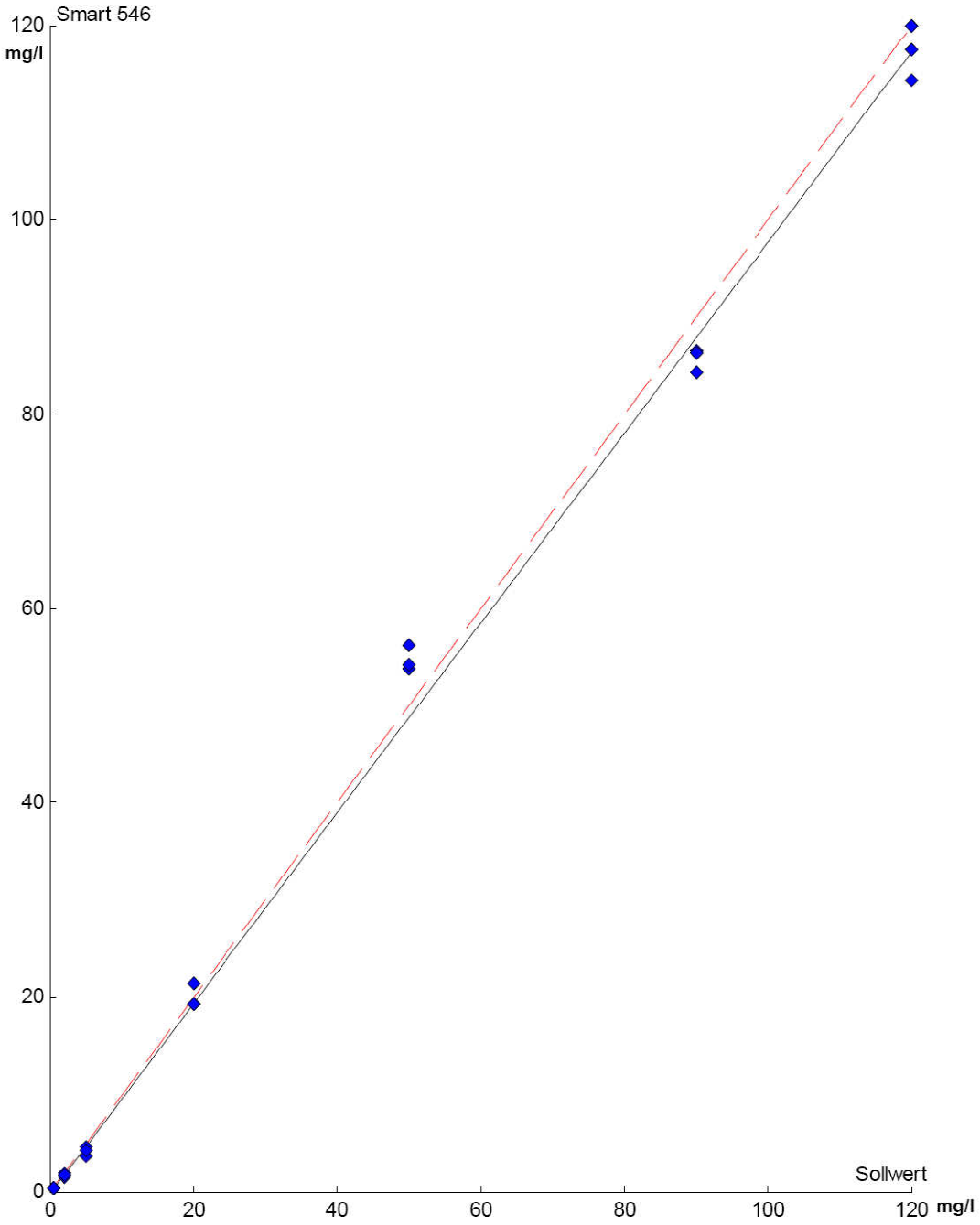

Fig. 7: Linearity test for serum samples (Smart 546) r = 0.998
Passing-Bablok agreement test N = 21
Slope: 0.979 [0.960 to 1.033]
Intercept: -0.19 [-0.57 to –0.08]

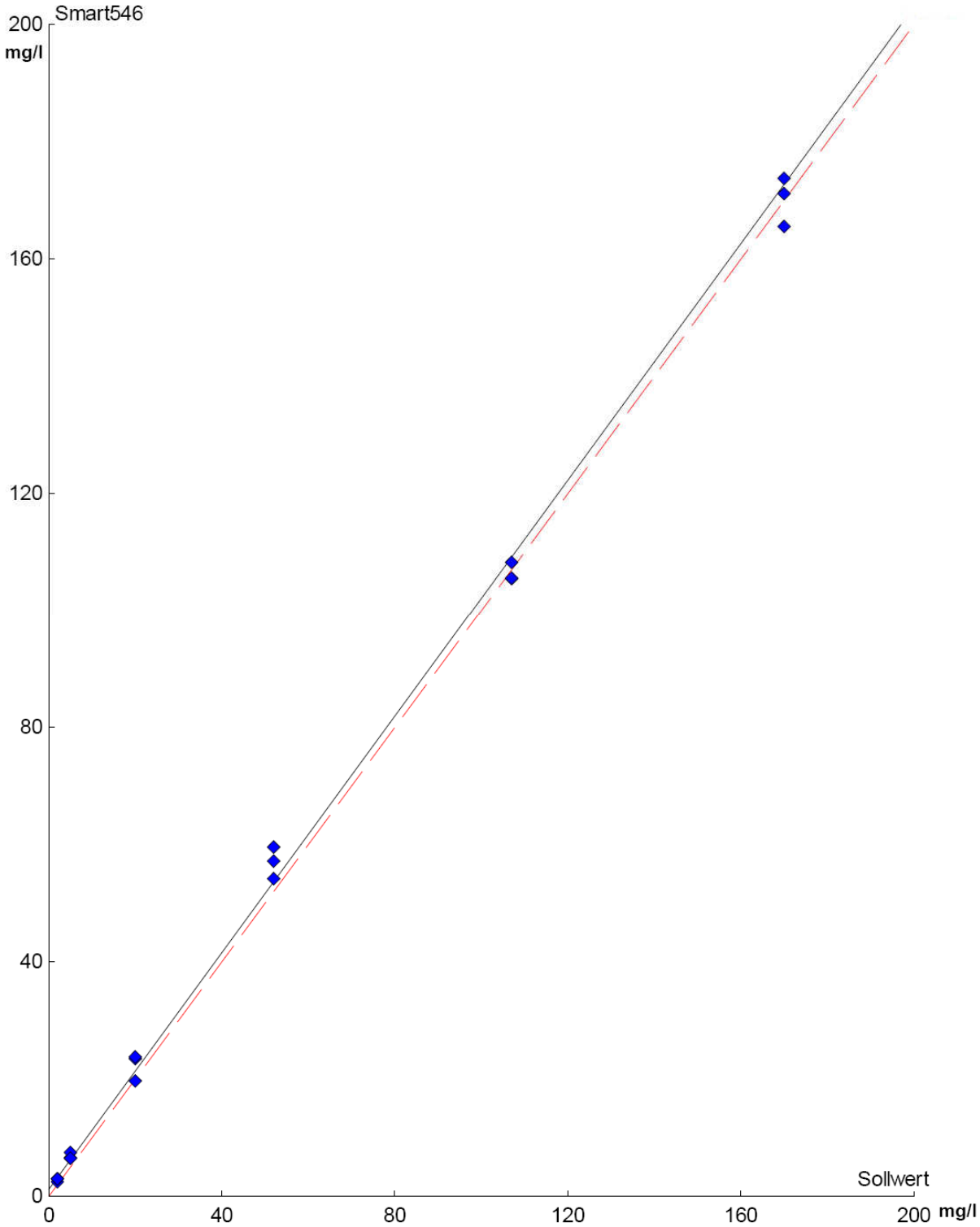

Fig. 8: Linearity test for whole blood samples (Smart 546) r = 0.999
Passing-Bablok agreement test N = 18
Slope : 1.009 [0.977 to 1.084]
Intercept : 1.22 [0.58 to 2.16]

7. Summary

The SMART 546 is a relatively compact tabletop unit that is designed for emergency situations, point-of-care concepts, and other possible applications with locally required laboratory diagnostics, to provide quantitative CRP/hsCRP values from whole blood as well as from serum / plasma, 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 SMART 546 measurement device, tested against the Cobas 6000 CRP measurement method as a reference, showed the best correlation between measurements in whole blood mode and the serum reference. The comparison of both serum ascertainment methods also showed acceptable correlation. The results of the SMART plasma measurements were significantly lower than the measured serum references. The SMART 546 can be considered an excellent POCT measurement device for ascertainment from whole blood and serum.

The precision of the SMART 546 is, as expected, higher for CRP 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. Using the new test kits, it is possible to measure with the Smart 546 in a concentration range of 0.4 mg/L to 173.8 mg/L, with the precision indicated above. Outside of the measurement range, <0.4 or >173.8 / >200 is given as a result.

During the evaluation phase described here, the charge of the test kits appeared to have the largest influence on the quality of the results. This appears to be significantly improved with the newest reagent generation from the manufacturer.

In summary, it can be determined that the SMART 546 has excellent practical applicability for the concepted application, and good measurement characteristics, especially for measurements with whole blood samples. We will therefore put the SMART 546 into use in our on-site labs, together with the blood count measurement device KX-21/Sysmex, in emergency diagnostics.

8. References

(1) Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, Part I. J Clin Chem Clin Biochem 1983;21:709-20

9. Appendix

Nr.	Proben-ID#	Cobas 6000	SMART 546	SMART 546	SMART 546
			(Serum-Modus)	*(Vollblut-Modus)*	*(Serum-Modus)*
				vor Zentrifug.	EDTA-Plasma.
		CRP [mg/L]	CRP [mg/L]	CRP [mg/L]	CRP [mg/L]
1	290001	131,98	118,0	128,8	97,9
2	290007	6,64	5,3	6,6	3,9
3	290008	30,71	28,8	27,8	18,0
4	290016	19,52	18,1	20,8	14,0
5	290019	13,53	12,2	15,1	9,1
6	290021	35,86	30,8	28,0	26,1
7	290022	11,44	12,3	12,8	9,0
8	290023	89,74	74,1	78,7	55,0
9	290024	64,72	58,7	60,2	48,3
10	290026	117,84	94,7	105	79,6
11	290338	5,89	4,1	5,8	3,7
12	290340	3,78	3,5	4,5	2,8
13	290341	5,67	5,3	6,1	4,0
14	290358	84,05	69,2	76,7	60,0
15	290362	11,32	9,0	11,6	7,8
16	290364	133,18	117,1	122,5	90,8
17	290365	10,30	10,5	10,1	8,3
18	290369	33,55	27,6	25,7	26,4
19	290371	42,69	33,5	44,2	30,5
20	290376	29,30	25,2	22,2	18,2
21	290886	7,77	5,7	7,5	5,3
22	290890	102,46	99,3	106,0	74,8
23	290891	7,45	6,8	6,6	5,5
24	290898	34,14	30,8	29,7	24,8
25	290900	35,10	30,0	32,0	29,0
26	290906	7,59	9,1	8,1	7,0
27	290910	29,49	25,2	25,5	19,1
28	290912	38,43	38,8	36,6	30,5
29	290914	5,97	6,6	6,3	5,5
30	290917	2,50	3,1	2,2	2,0
31	290919	3,65	3,8	3,7	3,2
32	290926	50,95	45,0	51,7	39,3
33	290930	102,65	104,7	110,8	88,3
34	290932	75,86	86,3	82,6	57,0
35	290934	69,83	68,0	63,7	58,0
36	290935	8,53	8,1	8,6	7,2
37	290937	8,86	9,3	9,3	7,1
38	290938	16,22	17,2	15,3	11,0
39	290939	30,94	27,7	30,7	22,5
40	290940	3,15	3,1	2,5	2,5
41	291392	74,16	69,8	66,0	64,2
42	291397	55,89	59,2	52,9	49,4
43	291402	8,98	8,5	9,5	5,8
44	291409	17,42	18,0	17,5	13,3
45	291414	7,26	7,0	6,9	6,1
46	291420	3,74	3,5	3,5	2,9
47	291429	12,52	12,6	11,6	9,1
48	291436	89,95	77,6	88,4	67,3
49	291437	80,23	66,9	62,7	56,7
50	291438	7,08	6,5	8,1	4,6

Appendix II: SMART 546 reagents used

LS Nr. / Datum	Lieferdatum	Menge	LOT-Nr.	Haltbarkeit	intern vergebene RFID-Kartennummer	Messung	Messdatum/ Kürzel Untersucher	Bemerkung
Sysmex 150943 / 14.05.2007	15.05.2007	2	**066296-3** (Karte 1) und **066296** (Karte 2)	31.03.2008	1 und 2	Einarbeitung und Orientierungsmessungen; Methodenvergleich	08.06.07/SH	
Sysmex 151243 / 15.05.2008	16.05.2007	5	**66296**	31.03.2008	3 und 4	Methodenvergleich	11.06.07/SH	4. Packung angefangen
Sysmex 152406 / 24.05.2007	25.05.2007	4	**66296**	31.03.2008		keine Messungen durchgeführt		keine Packung verwendet
Kein Lieferschein; Lieferung persönlich durch Herrn Gruber, Fa. EUROLyser	11.06.2007	4	**629901**	31.03.2008	5 bis 8	Methodenvergleich; Serum: Impräzision	12.06.07 /SH; 14.06.07/SH; 15.06.07/SH; 18.06.07/KR	Herr Gruber hat 8 vorhandene Packungen mitgenommen und 4 neue Packungen übergeben; grüne Beschriftung auf den Karten
EuroLyser 12.06.2007	13.06.2007	4	**629901**	31.03.2008	9 bis 12	Serum + Vollblut: Impräzision	18.06.07/KR; 19.06.07/KR; 20.06.07/KR*	*WH der Messungen / Vollblut am 26.06.07
Sysmex 155226 / 18.06.2007	19.06.2007	3	**66296-3**	31.03.2008	13 bis 15	Serum + Vollblut: Linearität Vollblut: Impräzision 100 mg/L	20.06.07/KR*; 22.06.07/KR*	*WH der Messungen / Vollblut am 26.06.07
EuroLyser 21.06.2007	22.06.2007	4	**629901-2**	31.03.2008	16 bis 17	Wiederholungsmessungen Vollblut: Impräzision ca. 100 mg/L und Linearität	26.06.07/KR	Restmenge: 2 Packungen ä 32 Testkits
EuroLyser 09.07.2007	10.07.2007	5	**629901-2**	31.03.2008	2.1 bis 2.6	Wiederholungsmessungen Methodenvergleich	16.07. - 22.07.2007/SH	Restmenge: 26 Testkits

Note

This document has been translated from German. You will find the original version of this document at http://www.eurolyser.com