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EVALUATION SMART Homocysteine

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Date: 23/06/2007

1. Scope and goals of the evaluation

1.1 Method comparison

Testing the correlation between the HCY measurement results in the SMART 340 analyzer from serum and plasma samples and the results of the HPLC method of the hospital in Bad Homburg-Germany.

1.2 Imprecision

Characterization of the precision of the SMART HCY test at 3 levels

1.3 Day/Day variation-Stability testing

2. Samples, reagent kits, and consumables

Samples:

Samples taken from the daily routine of 5 days (03/06/2007 to 05/06/2007), and deep frozen plasma samples from the routine lab of Bad Homburg's hospital.

Material and methods:

SMART 340 analyzer with the following serial number, have been taken from the current production lot:

SMART: SNO Aa0127

Reagent kits:

2 packages of article ST0110, with LOT no. 1001007-2, were taken from the current production charge.

QC materials

To check the correctness, the QC kit provided by IRIS Technologies International, Ltd. Clinchek 1 and Clinchek 2, was used.

3. Test processing with the SMART system:

The reagent kit contains all the materials required to carry out the tests.

Warm up cuvettes at least 10 minutes at room temperature

Pipette sample with SMART Pipette into SMART Cuvette

Apply ERS Cap and place cuvette into analyser-start measurement

4. Method comparison:

The method comparison was carried out with 20 samples from the pool of the hospital “Krankenhaus Bad Homburg”, in the range of 8,26 and 44,5 µmol/l HCY.

The R² value of the linear regression was determined, as well as the k and d value, according to the formula $y=kx+d$ (y =SMART HCY and x = HPLC) Measurements have been performed in Bad Homburg directly by Dr. Volker Schlüter. (Biomed)

The chart and raw data are in the appendix.

Sample	HCY HPLC	HCY SMART	Sample Material
1	40,4 µmol/l	32,5 µmol/l	Plasma frozen
2	35,1 µmol/l	33,8 µmol/l	fresh serum
3	44,5 µmol/l	37,4 µmol/l	fresh serum
4	8,6 µmol/l	6,9 µmol/l	fresh serum
5	8,3 µmol/l	6,7 µmol/l	fresh serum
6	16,5 µmol/l	15,8 µmol/l	fresh serum
7	12,1 µmol/l	16,3 µmol/l	Plasma frozen
8	18,3 µmol/l	22,2 µmol/l	Plasma frozen
9	19,4 µmol/l	22,4 µmol/l	Plasma frozen
10	38,4 µmol/l	33,4 µmol/l	fresh serum
11	17,8 µmol/l	17,8 µmol/l	fresh serum
12	8,3 µmol/l	8,7 µmol/l	fresh serum
13	13,8 µmol/l	15,1 µmol/l	fresh serum
14	17,0 µmol/l	16,8 µmol/l	fresh serum
15	12,8 µmol/l	13,7 µmol/l	fresh serum
16	34,0 µmol/l	29,4 µmol/l	Plasma frozen
17	30,9 µmol/l	26,0 µmol/l	Plasma frozen
18	26,0 µmol/l	23,6 µmol/l	fresh serum
19	18,0 µmol/l	23,4 µmol/l	Plasma frozen
20	27,0 µmol/l	26,3 µmol/l	fresh serum

Table I:
 HCY values for fresh serum and frozen plasma from SMART, compared with HPLC

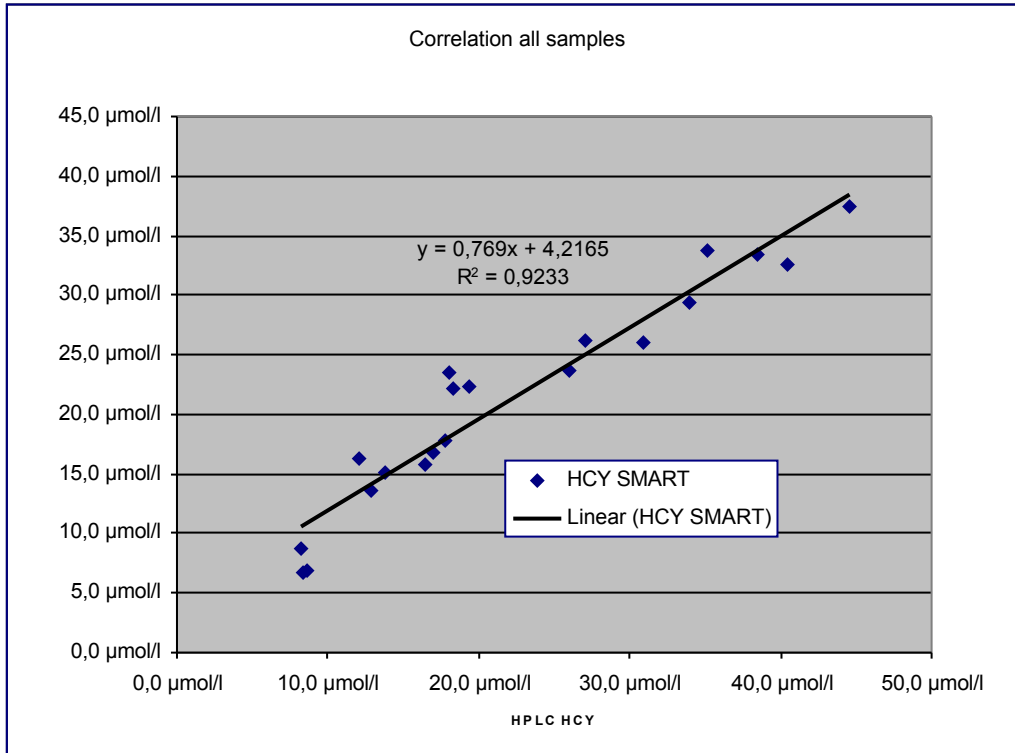
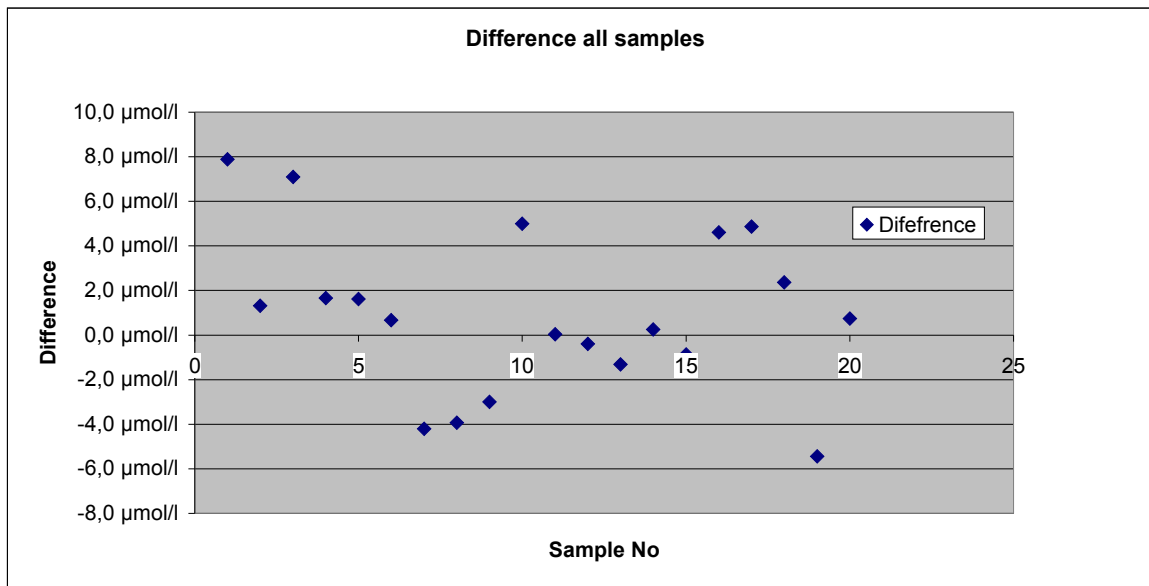


Chart I:
Correlation between SMART HCY (y) and HPLC (x)
 $R^2=0.9233$ $Y=kx+d=0,769+4,2165$



Difference plot for all samples (serum and frozen plasma)

Sample	HCY HPLC	HCY SMART	Sample Material
2	35,1 µmol/l	33,8 µmol/l	fresh serum
3	44,5 µmol/l	37,4 µmol/l	fresh serum
4	8,6 µmol/l	6,9 µmol/l	fresh serum
5	8,3 µmol/l	6,7 µmol/l	fresh serum
6	16,5 µmol/l	15,8 µmol/l	fresh serum
10	38,4 µmol/l	33,4 µmol/l	fresh serum
11	17,8 µmol/l	17,8 µmol/l	fresh serum
12	8,3 µmol/l	8,7 µmol/l	fresh serum
13	13,8 µmol/l	15,1 µmol/l	fresh serum
14	17,0 µmol/l	16,8 µmol/l	fresh serum
15	12,8 µmol/l	13,7 µmol/l	fresh serum
18	26,0 µmol/l	23,6 µmol/l	fresh serum
20	27,0 µmol/l	26,3 µmol/l	fresh serum

Table 2:
 HCY values for fresh serum only

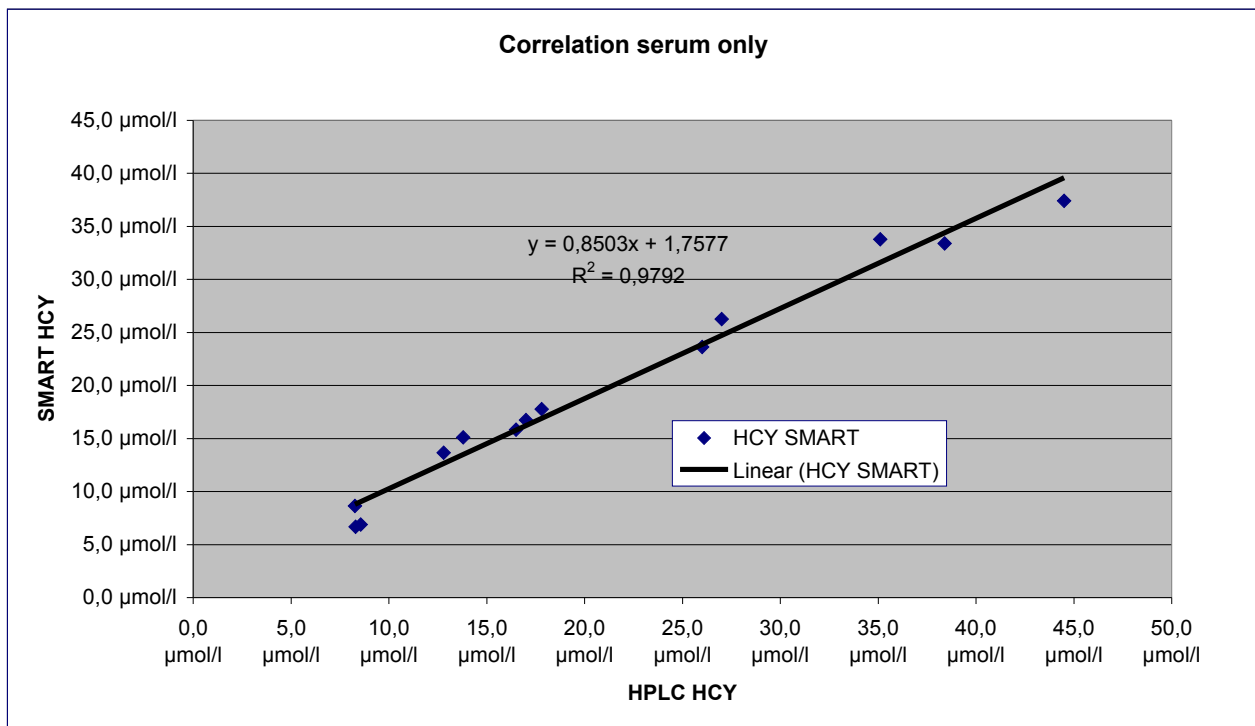


Chart 2:
 Correlation between SMART HCY (fresh serum only) (y) and HPLC (x)

$R^2=0.9792$
 $Y=kx+d = 0.8503x+1.7577$

Difference Plot :

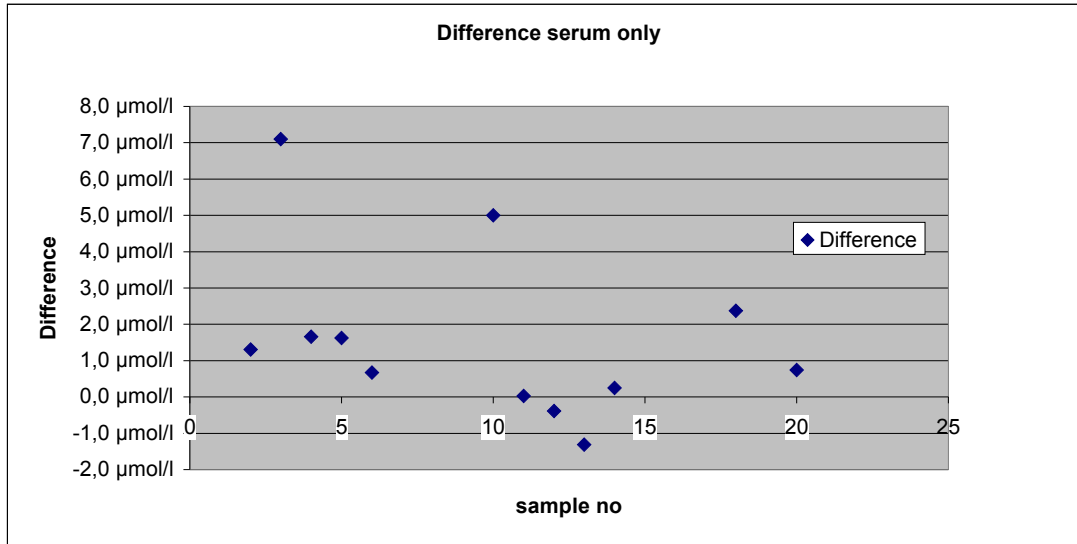


Chart 3:
Difference plot, for serum samples only

5. Imprecision:

The imprecision of the SMART HCY was determined with 3 different patient serum samples (low-mid high, each sample was tested 12 times).

Sample	No12	No11	No20
1	8,3 µmol/l	17,8 µmol/l	27,0 µmol/l
2	8,8 µmol/l	15,0 µmol/l	28,6 µmol/l
3	7,8 µmol/l	16,0 µmol/l	27,1 µmol/l
4	8,0 µmol/l	17,0 µmol/l	27,2 µmol/l
5	8,1 µmol/l	18,8 µmol/l	28,1 µmol/l
6	8,2 µmol/l	17,3 µmol/l	29,1 µmol/l
7	8,4 µmol/l	18,2 µmol/l	29,0 µmol/l
8	8,9 µmol/l	18,1 µmol/l	28,9 µmol/l
9	7,6 µmol/l	17,8 µmol/l	26,5 µmol/l
10	7,4 µmol/l	17,9 µmol/l	27,7 µmol/l
11	8,8 µmol/l	17,4 µmol/l	27,8 µmol/l
12	8,9 µmol/l	17,5 µmol/l	27,5 µmol/l
Mean	8,3 µmol/l	17,4 µmol/l	27,9 µmol/l
Stabw	0,5 µmol/l	1,0 µmol/l	0,8 µmol/l
cv	5,95%	5,64%	2,98%

Table 3: within run precision

6. Day to day variance with Diazyme HCY Controls low and high

Stability test		day 0	day2	day5	day7	day14	day21
6/22/2007	hcy	result	result	result	result	result	result
HRc100207	7.0	7.0	5.7	6.4	6.0	6.4	6.2
HRc300207	29.0	31.0	26.5	31.0	31.7	31.3	28.7

7. External QC material:

The Clincheck 1 and Clinscheck2 control serum was analysed on both HPLC and SMART analyser.

QC Material	HPLC	SMART
ClinChek1	9,41	8,97
ClinChek2	21,7	23,82

Order No: 23080/Lot No: 626/ Exp: 06.2009

Supplier: Recipe Chemicals Instruments D-80335 Munich

ClinCheck 1 Control range for LC-MS/MS 8,08-12,1 $\mu\text{mol/l}$ (target 10,1)

ClinCheck 2 Control range for LC-MS/MS 18,8-28,2 $\mu\text{mol/l}$ (target 23,5)

8. Summary:

The direct enzymatic method used in the SMART analyzer has a good correlation to HPLC methodes.

As fresh serum samples correlates better then frozen plasma samples, fresh serum should be defined as sample material of the first choice.

The precision is acceptable for a Point of Care system with $cv=2,98\%$ $cv=5,64\%$ and $cv=5,95\%$ for high medium and low HCY concentrations.

External QC material values have been confirmed closed to target values for low and high controls.

Salzburg 23/06/2007

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