Table 1. Overall performance of laboratories on 14 different competency assessment exams and performance of 10 laboratories on the safety examination.

A. Overall performance

Topic	No. of exam takers	No. of laboratories	Test scores		
			Mean	Median	Range
Safety	1868	111	86.8	90	30–100
Specimen processing	1357	101	91.9	90	30–100
Phlebotomy	1269	99	92.0	90	40–100
Urinalysis	1225	97	90.9	90	20–100
Hematology	975	91	87.2	90	20–100
Chemistry	818	95	80.1	80	10–100
CSF[a]/Serous fluid	813	87	91.6	90	20–100
Coagulation	759	87	88.3	90	10–100
Gram stain	670	91	81.0	90	0–100
Transfusion	464	76	71.8	70	10–100
Toxicology	418	56	76.7	80	10–100
Microbiology	418	79	78.5	80	30–100
Hepatitis and HIV AB	294	60	91.1	90	50–100
Blood donor	214	61	80.0	80	10–100

B. Results for 10 laboratories

Laboratory code[b]	No. of exam takers	Test scores		
		Mean	Median	Range
1-a	79	96.0	100	80–100
1-b	122	95.0	100	70–100
2	93	90.4	90	80–100
3	60	89.8	90	70–100
4	80	88.8	90	70–100
5-a	58	86.0	90	50–100
6	89	83.6	90	50–100
7	119	82.9	80	40–100
5-b	66	82.6	80	40–100
8	55	82.5	80	40–100

[a] CSF, cerebrospinal fluid; AB, antibodies.

[b] Institutional names were replaced with random numbers. Unique laboratories within the same integrated health system have identical numbers and are differentiated using "a" and "b".

subscribing to the competency assessment service, and subscriptions cost $400–1000 annually depending on the size of the subscribing institution. There is no easy way around this limitation because this University-based endeavor must be self-supporting. Another limitation is that benchmark examination scores (Table 1A) are useful for relative, but not absolute, comparisons. We do not know whether the difference in mean scores across the 14 topics is attributable to differences in the topics or differences in the examinations. Thus, in establishing goals for improving the quality of performance in a particular topic, laboratory supervisors should seek to improve the competency of their laboratories relative to the national means or the median scores.

Future improvements to the competency assessment system will focus on expanding the number of topics to be covered and the number of subscribing institutions. In the upcoming year, we will be adding two topics: patient safety and protein electrophoresis. The future direction for our competency assessment research is to perform an item analysis for each topic to determine which concepts within a topic are most problematic for laboratories. Laboratory supervisors should find these data valuable when planning their educational activities.

In summary, we have implemented an online competency assessment system that is now used as a supplement to traditional competency assessment methods by more than 200 laboratories across the US. We are hopeful that the results generated by the system will be used in quality improvement projects that increase competency in many areas of laboratory medicine.

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Total Plasma Homocysteine Measured by Liquid Chromatography–Tandem Mass Spectrometry with Use of 96-Well Plates, *Torsten Arndt,* Brunhilde Guessregen, Axel Hohl, and Bernd Heicke* (Bioscientia GmbH, Konrad-Adenauer-Strasse 17, D-55218 Ingelheim, Germany; * author for correspondence: fax 49-6132-781-428, e-mail torsten.arndt@bioscientia.de)

Increased total plasma or serum homocysteine is regarded as a risk factor for occlusive arterial or venous disease [reviewed in Refs. *(1, 2)*]. Homocysteine is measured by HPLC *(3)*, immunoassays *(4)*, or liquid chromatography–mass spectrometry *(5–9)*. We were faced with a large workload of ~200 samples/day, plus high material costs for commercially available immuno- and HPLC assays and laborious sample pretreatment for our HPLC appli-

cation. We thus wished to establish a high-throughput liquid chromatography–tandem mass spectrometry (LC-MS/MS) method. We aimed at a 96-well plate format for sample pretreatment and autosampling without protein precipitation, centrifugation, or derivatization steps.

We pipetted 50 μL of NaF-plasma, calibrator, or quality-control sample into each well of a 96-well plate (well volume, 2.2-mL) and added to each well 1000 μL of 0.4 μmol/L homocystine-d_8 (3,3,3′,3′,4,4,4′,4′-d_8-homocystine; Cambridge Isotope Laboratories). The plate was sealed with Parafilm (the tightness was tested with colored solutions) and mixed by vigorous shaking. Of this mixture, 50 μL was transferred to each well of a 96-well plate (well volume, 1.2 mL), diluted with 500 μL of dilute NaOH (50 μL of 6 mol/L NaOH in 500 mL of water), and reduced by the addition of 25 μL of 200 mmol/L dithiothreitol (threo-1,4-mercapto-2,3-butandiol; Aldrich). The plate was sealed (see above), shaken to mix the contents of the wells, left at room temperature for 15 min, and again shaken. We removed the Parafilm and placed the 96-well plate in the autosampler (10 °C) for overnight LC-MS/MS analysis. The sample dilution was 1:241.5 (~1:240 as stated throughout this report).

The LC-MS/MS system consisted of a Surveyor MS pump and Autosampler combined with a triple-quadrupole TSQ Quantum mass spectrometer (Thermo Electron). The autosampler injected 2 μL (~0.008 μL of the native plasma sample) into the system. The mobile phase for the chromatography was a mixture of 2 mL/L acetic acid and methanol (50:50 by volume), the flow rate was 0.2 mL/min, run time was 2 min, and the column was a ThermoHypersil BetaMaxAcid [100 × 2.1 mm (i.d.); 5-μm bead size] with a ThermoHypersil Drop-in Guard Cartridge precolumn (Thermo Electron). The mass spectrometer was operated in the electrospray atmospheric pressure ionization, positive ion, and single-reaction-monitoring mode. The collision energy was 12 eV. Ion transitions *m/z* 136→90 (homocysteine) and 140→94 (homocysteine-d_4), both attributable to a neutral loss of formic acid *(6)*, were assessed. The LC-Quan software (Thermo Electron) was used for calculating the homocysteine/homocysteine-d_4 peak-area ratios and quantification of homocysteine. Calibration, quality control, and checks for carryover were performed daily. The details and typical chromatograms obtained by our method are given in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol50/issue4/.

Passing and Bablok regression *(10)* was used for unbiased method comparison and linear regression for calculating the calibration functions. The significance of differences between mean and median homocysteine concentrations for different groups was tested by parametric ANOVA (means) and nonparametric Kruskal–Wallis ANOVA (medians). All calculations were done with the Analyze-it software for Microsoft Excel (Analyze-it Software Ltd.). The significance level was set at 95%, or $P = 0.05$.

Calibration curves were constructed with aqueous homocysteine solutions and with homocysteine added to human plasma. Both procedures were found to be not statistically different (see the online Data Supplement). The validation of our procedure included comparison of results obtained with use of 1.2-mL glass vials and the 96-well plates, optimization of the plasma dilution, and evaluation of the stability of the pretreated plasma samples over 3 days. Passing and Bablok regression (1.2-mL glass vial vs 96-well plate format and 1:240 vs 1:570 plasma dilution), box-plot analysis for testing the effects of sample storage of up to 3 days after sample pretreatment, parametric ANOVA, and nonparametric Kruskal–Wallis ANOVA did not detect significant differences between the corresponding sample pretreatment protocols. This confirmed the comparability of the 96-well plate sample pretreatment with the commonly used 1.2-mL glass vial format, of the 1:570 plasma dilution with the 1:240 plasma dilution protocol, and the stability of the pretreated plasma samples over (at least) 3 days (see the online Data Supplement).

The comparability of our LC-MS/MS method with HPLC (Immundiagnostics) was tested with 187 plasma samples. The Passing and Bablok correlation function (Fig. 1A) was as follows: LC-MS/MS = 1.12 × HPLC + 0.41 μmol/L. The 95% confidence intervals were 1.075–1.162 for the slope and −0.049 to 0.856 μmol/L for the intercept; the slope was thus statistically significantly different from 1. Bland–Altman analysis (Fig. 1B) yielded a difference of 1.81 (95% confidence interval, 1.58–2.04). Thus, our LC-MS/MS gave significantly higher (~15–18%) homocysteine results than the HPLC method. Similar results, pointing to the need for a true homocysteine standard *(11)*, were reported by Nelson et al. *(8)* and, for a comparison between LC-MS/MS and the Abbott IMx assay, by McCann et al. *(9)*.

Significant reductions in material and personnel costs were reported by McCann et al. *(9)* when they used LC-MS/MS instead of HPLC for homocysteine measurement. This was confirmed by our calculations. Material and personnel costs were diminished by 90% and 60%, respectively. The analysis time was reduced to 2 min/analysis or 400 min/200 samples with our LC-MS/MS method, instead of 12–15 min/analysis or 2400–3000 min/200 samples for HPLC. Earlier reports on homocysteine analysis by LC-MS/MS did not address HPLC column life or LC-MS/MS system maintenance, although both can be important parts of the total analysis costs. Our large sample dilution gave a HPLC column life of ~10 000 analyses and LC-MS/MS maintenance intervals of ~4 months. We consider this an important part of the analysis cost reduction. Most importantly, the 17- to 80-fold greater plasma dilution and the ~37-fold smaller injection volume (~0.008 μL of native plasma by our method) compared with earlier reports *(5–9)* minimized the matrix load with each injection and thus the ion suppression, despite our exclusion of protein precipitation. A greater plasma dilution (1:570-fold) did not give increased homocysteine and homocysteine-d_4 signals, confirming that there was no ion suppression with the 1:240-fold sample dilution protocol under routine conditions. Such an ex-

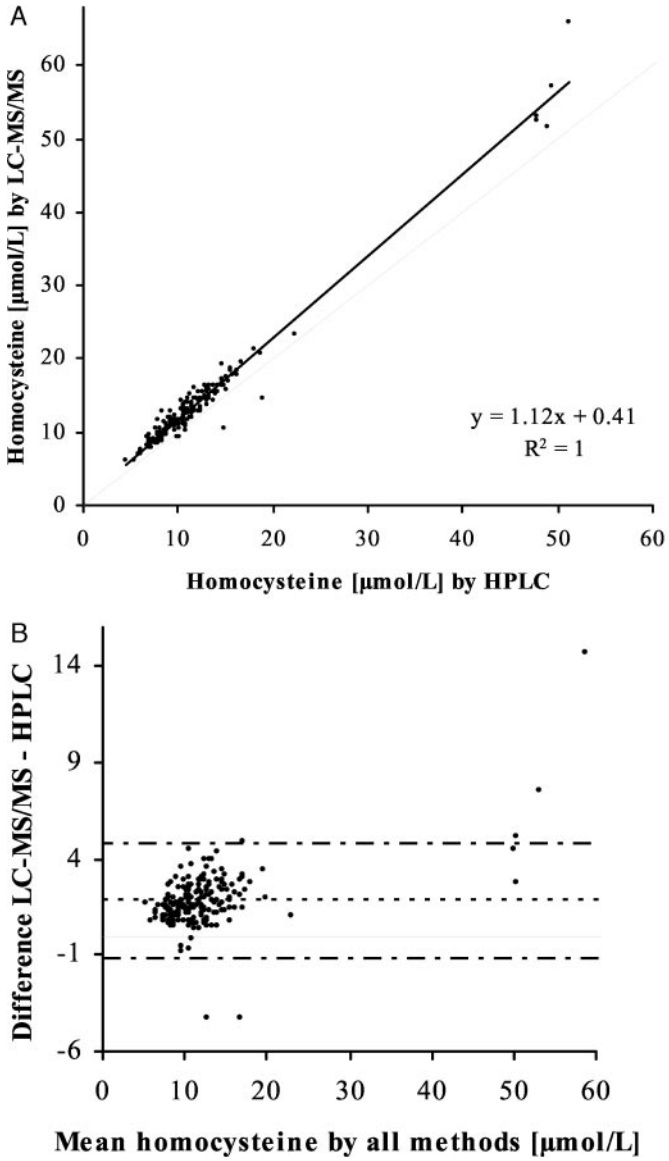

Fig. 1. Passing–Bablok regression analysis (*A*) and Bland–Altman difference plot (*B*) for a commercially available HPLC assay and our LC-MS/MS method for total plasma homocysteine.

periment might be a valuable alternative to the procedure described by Streit et al. *(12)* when analyte-free samples are not available.

Our method is specific, sensitive, reproducible, and accurate (see Table 1 of the online Data Supplement). A 96-well plate format sample pretreatment in combination with LC-MS/MS for homocysteine analysis has been described previously *(7, 9)*. We consider the combination of this format with a large plasma dilution without deproteinization for high-throughput homocysteine analysis the most important aspect of our application.

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Diminished Urinary Free Cortisol Excretion in Patients with Moderate and Severe Renal Impairment, *K.C. Allen Chan,[1] Lydia C.W. Lit,[1] Eric L.K. Law,[1] Morris H.L. Tai,[1] C.U. Yung,[2] Michael H.M. Chan,[1] and Christopher W.K. Lam[1]** ([1] Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong Special Administrative Region, China; [2] Department of Medicine, Alice Ho Miu Ling Nethersole Hospital, Tai Po, New Territories, Hong Kong Special Administrative Region, China; * address correspondence to this author at: Department of Chemical Pathology, Chinese University of Hong Kong, Prince of Wales Hospital, 30-32 Ngan Shing St., Shatin, New Territories, Hong Kong SAR, China; fax 852-2636-5090, e-mail waikeilam@cuhk.edu.hk)

The diagnosis of Cushing syndrome remains a challenge for most general clinicians and even endocrinologists. Because the clinical features of Cushing syndrome overlap with those in some healthy obese individuals, biochemical investigations play an important role. The 24-h urinary free cortisol excretion is widely used because of its relatively good sensitivity and specificity *(1, 2)*. Al-