S/N ratio (Figure 1B and D). Because $(NH_4)H_2PO_4$ is potent alkali cation adduct ion suppressor (3), these outcomes indicate that the SPA of both sources might contain significant amounts of alkali cations.
The background of this phenomenon is that proteins in MALDI-TOF MS and thus also in SELDI-TOF MS analyses generally are ionized through protonation, i.e. formation of a $[M+H]^+$ ion. In addition to protonation also alkali cationization can occur, in which alkali cations such as Na^+ and K^+ are bound instead of a proton, i.e. formation of $[M+Na]^+$ and $[M+K]^+$ ions. Both processes are in competition of each other and may result in extra peaks in the mass spectrum. As the mass resolving power of the PBS IIc analyzer is insufficient to separate these extra peaks from that of the $[M+H]^+$ ion, the overall result of the presence of alkali cations is peak broadening and decrease of peak intensity, i.e. deterioration of RMA and decrease of S/N ratio.
The addition of NaCl and KCl to SPA-c significantly deteriorated the RMA of the signals of both proteins, of which the decline could be compensated by the co-addition of $(NH_4)H_2PO_4$ as an alkali cation adduct ion suppressor (Figure 1A and C). The overall effect on the S/N ratio showed a trend of improvement when $(NH_4)H_2PO_4$ was added (Figure 1B and D).

Conclusions

The relative mass accuracy and signal-to-noise ratio of the signals as obtained by the SELDI-TOF-MS analysis of apomyoglobin and albumin were deteriorated by controlled addition of alkali cation salts to SPA and improved by addition of an alkali cation adduct ion suppressor. Thus, non-controlled presence of alkali cation ions in the EAM could affect the reproducibility of SELDI-TOF MS analysis.

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Standardization of calibration and quality control using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry

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Proteomic pattern analysis by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) is one of the most promising new approaches for the discovery and identification of potential biomarkers for various types of cancer, e.g., ovarian (1), prostate (2), lung cancer (3) or for other diseases, like inflammatory diseases (4). Notwithstanding using identical types of biological specimens and the same analytical platform (5), several groups identified different patterns for the same types of cancers. Differences between the pre-and post-analytical strategies are responsible for the different results (6). Therefore, a stringent standardized protocol is needed, not only for pre- and post-analytical aspects, but also for calibration and quality control (QC) performance.
The aim of our study was to establish a well-defined protocol for calibration of the Protein Biosystem IIc (PBS IIc) instrument (Ciphergen Biosystems Inc. Fremont, CA, USA), to implement QC samples with independent certified standards and to determine acceptance criteria for the QC samples. Because the QC samples were spotted on a NP-20 array (Ciphergen Biosystems Inc.), which is a normal phase array, without washing or selective binding steps, only the MALDI-TOF MS part of the PBS IIc instrument was checked.

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Methods

Calibration samples
Instrument calibration was performed externally with the All-in-1 peptide and All-in-1 protein standards. Both standards as well as the SPA solution as energy absorbing matrix (Ciphergen Biosystems, Inc.) were prepared according to the recommendations of the manufacturer, with the exception that TFAH as a solvent component was used instead of TFA. On a

NP-20 array with 8 spots, 1 µL of All-in-1 peptide standard was applied to the spots A-D, while 1 µL of All-in-1 protein standard was applied to the spots E-H. After preparation, the same calibration array was used for all experiments during the whole period, using one spot each week. The spots, including within-spot positions were alternated weekly. A standard protocol was used to generate the peptide, protein-low and protein-high calibration equations (7).

Quality control sample

The QC samples consisted of the Proteomass MALDI MS standards insulin and apomyoglobin, and albumin (Sigma-Aldrich CO, St. Louis, MO, USA). According to the specifications the standards should produce in MALDI-TOF MS analysis the $[M + H]^+$ ions at *m/z* 5734.51, *m/z* 16,952.27 and 66,429.09 for insulin, apomyoglobin and albumin, respectively. The QC samples were prepared according to the manufacturer's specifications. Insulin and apomyoglobin were mixed together as one QC sample and albumin was used as the second QC sample. The insulin/apomyoglobin QC samples were diluted 5 times with 1% of TFAH solution. For the albumin QC samples there was no additional dilution. Five µl of both QC samples were applied on a NP-20 array. SPA was prepared as described above. The array was dried and afterwards 1 µL SPA solution was applied. The spotting of SPA was repeated once. Each batch of the QC sample was divided into aliquots and stored at –20 ºC. A batch was used for one month, according to manufacturer's specifications and aliquots were spotted every week on a new spot of a NP-20 array.

Instrumental settings and calibration

The calibration and QC arrays were analyzed on a PBS IIc instrument. Different settings like, high mass setting, optimization range, focus mass, deflector, laser intensity and deflector sensitivity, were used for the peptide, protein low and protein high standards and both QC samples (7). The calibration curves were generated with the 3-parameter calibration in the Biomarker ProteinChip Software 3.2.0 (Ciphergen Biosystems Inc.). The *m/z* values of insulin, apomyoglobin, and albumin were determined after calibrating with the most recent peptide, protein-low, and protein-high calibration equations, respectively.

Data analysis

Data analysis was performed with in house developed software (ShewhartPlots), which was based on the Shewhart control chart principle (8). The following parameters were imported in the software; *m/z* values, intensities, signal-to noise (S/N) ratios and peak resolutions. Two dimensional Youden plots were made by drawing insulin (x-axis) and apomyoglobin (y-axis) in one plot for all parameters and three dimensional Youden plots were made by drawing insulin (x-axis), apomyoglobin (y-axis) and albumin (z-axis) in one plot for all parameters. The fulfilment of the following Westgard rules was checked: 1_{3s}, 2_{2s}, 4_{1s}, 8_x, 10_x, and 12_x (9).

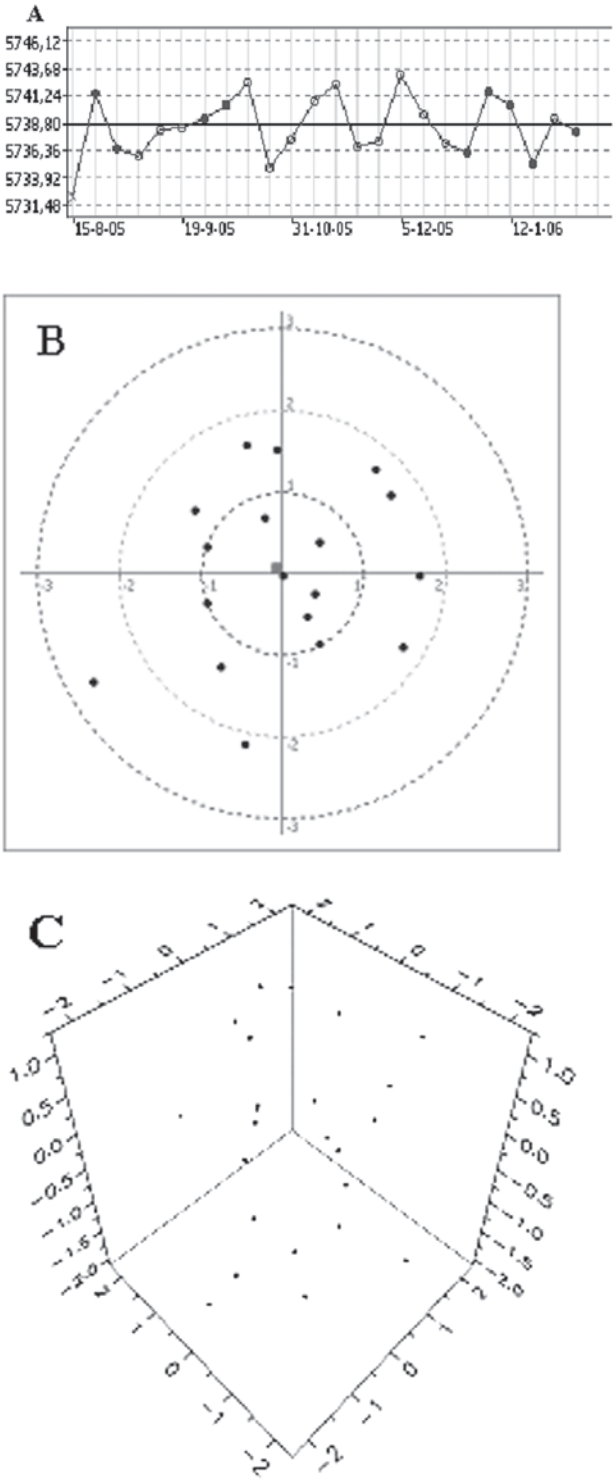

Figure 1. Examples of a graphic and Youden plots for the *m/z* values, S/N ratios and peak resolutions generated with the in house developed Shewhart plots. The process mean and the standard deviation (SD) values (+ 1, 2 and 3 × SD, -1, 2 and 3 × SD) of the *m/z* values of insulin are indicated (A). The *m/z* values are indicated on the x-axis and the SD values are indicated on the y-axis. The two dimensional Youden plot of the S/N ratios of insulin and apomyoglobin are illustrated (B). The S/N ratios of the insulin and apomyoglobin are indicated on the x- and y-axis, respectively. The SD values (+ 1, 2 and 3 × SD, -1, 2 and 3 × SD) are indicated on the x- and y-axis. The three dimensional Youden plot of the peak resolutions of insulin, apomyoglobin, and albumin are shown (C). The peak resolutions of insulin, apomyoglobin, and albumin are indicated on the x-, y-, and z-axis, respectively. The SD values are indicated on the different axes.

Results

After measuring the QC samples for a longer period, we concluded that most data points were within the process mean ± 2 standard deviations (SD) and none of the points were outside the process mean ± 3 SD range. On the basis of those results, we defined the following acceptance criteria, data points should be in the established range of the process mean ± 2 SD for the *m/z* values, peak intensities, S/N ratios, and peak resolutions for insulin, apomyoglobin and albumin in the QC samples. Figure 1 shows a graphic and a two and three dimensional Youden plot generated with the software.

In this study was demonstrated that variations in the signal of the QC samples can be caused by pipetting variability in the handling of the QC sample, spot and chip variability, crystallization of the energy absorbing matrix, and laser detector variability over time.

Conclusions

Any new technology, particularly one being presented as a potential clinically used diagnostic tool, requires stringent quality control to evaluate analytical performance over time. Instrument performance, however, must be compared not only during one experiment, but also over the course of time. In this study a standard protocol for calibration was defined and acceptance criteria for the independent certified QC samples were established. The composition of the QC samples was based on the way of calibration. Insulin, apomyoglobin, and albumin were chosen to validate the peptide and protein-low calibration equations, respectively. By checking the calibration every week, the QC procedure acts prospectively, while in some studies the quality control acts retrospectively by including the QC samples in the profiling experiments and in some studies there is no quality control procedure described at all. Stringent QC prevents unreliable data acquisition from the very start and when the data of the QC samples exceed the acceptance criteria, actions needs to be undertaken before starting new protein profiling experiments.

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Influence of cytochrome P450 3A5 and multidrug resistance-1 gene single nucleotide polymorphisms (SNPs) on the tacrolimus area under the curve (AUC) in renal transplant recipients

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Tacrolimus is used worldwide for primary immunosuppression following renal transplantation. Moreover, tacrolimus has a narrow therapeutic index, which makes close therapeutic drug monitoring necessary to prevent both sub-therapeutic blood levels and toxic blood levels. Sub-therapeutic tacrolimus blood levels increase the risk of transplant rejection (1, 2), while toxic tacrolimus blood levels may lead to severe side effects such as nephrotoxicity and neurotoxicity (3). The high interindividual variability in tacrolimus pharmacokinetics complicates the realization of this narrow therapeutic index.

Therapeutic drug monitoring of tacrolimus can be performed in the most exact way by making 12-hour pharmacokinetic profiles. Recent studies (4, 5) showed

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