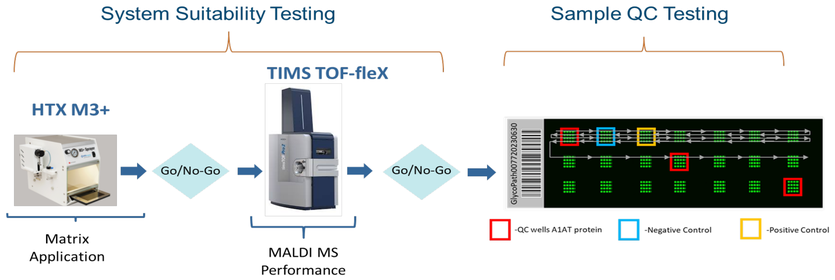
A Quality Management System Dashboard for Bruker's new GlycoTyper™ Targeted High-Throughput Glycoproteomics Platform

Grace Grimsley, Gray Huffman, and Klaus Lindpaintner

Glycobiology Solutions, Bruker Scientific Inc. 40 Manning Road, Manning Park, Billerica, MA 01821, USA

Introduction

As an integral part of our glycomics biomarker platform, which uses MALDI imaging MS as a glycan profile readout, we developed a quality management system (QMS) that comprehensively covers the entire workflow from sample preparation to data acquisition. This QMS includes multiple system suitability measurements of matrix spraying and instrument performance as well as on-slide QC and calibration samples that are integrated with test sample analysis.



Methods

System Suitability:

M3+ Sprayer System:
Pump flow rate is proportional to matrix density or enzyme coating.
Method: 50% ACN at 100 µL/min for 3 minutes = 300 µL or by weight = 271.6 µg (d=0.0.9054 g/mL (25ºC))
Acceptance: Collected volume within two standard deviations of the historic mean.

TIMS TOF fleX:
The MS system-suitability slide features 252 samples. Each sample is composed of 0.2 pMol of tryptically digested BSA (NEB, P8108S) printed by a Scienion S3 piezo-driven, non-contact dispensing system as a 22 nL spot.
Method: Samples are acquired in tissue imaging mode using 3500 shots across a 150 µm x 150 µm area within a 300 - 3000 Th mass range. The centroided ion intensities from three mass ranges (300-1000; 1000-2000; 2000-3000) are summed. The sample spot is chosen randomly from all previously nonacquired positions on the slide. Data is analyzed in real time and plotted in the System Suitability Dashboard (Figure 1).
Acceptance: Summed ion intensities must be within two standard deviations of historic mean.

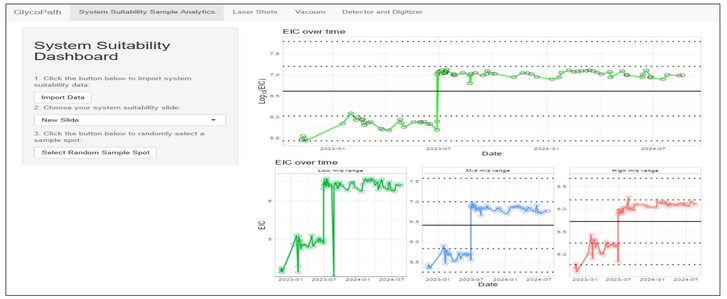


Figure 1: MALDI MS QMS Dashboard.

The system suitability tab for the TIMS TOF is shown in Figure 1. A plot of the historical performance of all samples (Log EIC) and acceptance criteria of +/- two standard deviations of the historical mean (dashed lines) is captured in the upper right of the panel.

Results

System Suitability Analysis:

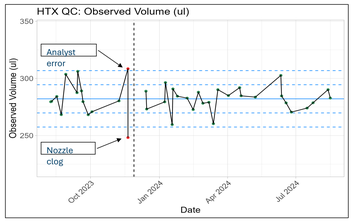


Figure 2: Detecting out-of-specification condition on the HTX sprayer

Figure 2 shows a snap-shot of the historical performance of the M3+ sprayer. The red dot highlights an out-of-specification measurement due to matrix build-up in the sprayer nozzle. After flushing the nozzle with solvent, the performance was restored.

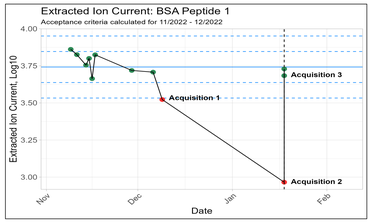
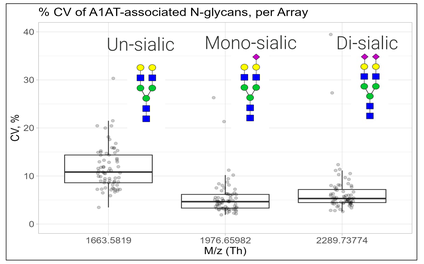
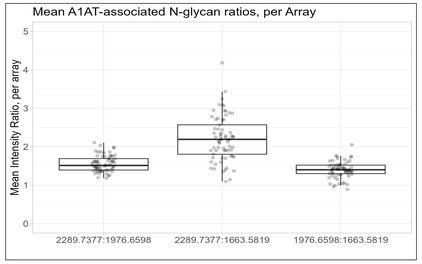


Figure 3: Detecting out-of-specification condition on the timsTOFfleX platform

Figure 3 tracks successive system suitability sample analyses showing a downward trend in the EIC. Acquisition 1 initiated a cleaning of the system, Acquisition 2 was collected post-cleaning, but indicated there was a build-up of charge on the optics. Once remedied, Acquisition 3 shows the system was back within specifications.

QC Slide Arrays: the following QC data are from a pilot N-glycan urine study of Lupus patients and healthy volunteers.





Figures 4, 5: Coefficient of Variation for A1AT glycans (Figure 4) and Glycan Ratios (Figure 5)

The % CV of three A1AT glycans per array for all arrays (n=69) is presented in Figure 4. Our goal was to achieve a QC coefficient of variation (CV) below 15%.
The A1AT QC data arrays were further explored to answer questions about sialic acid stability due to their ability to undergo in-source fragmentation (Figure 5).

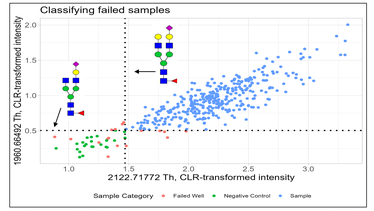


Figure 6: Clinical sample acceptance criteria

Where there is insufficient protein in the patient's urine or loss of capture-antibody binding activity, no or low glycan ion detection over the background will be observed and will be classified as a failed sample (Figure 6).

Conclusions

- A practical quality control strategy for the GlycoTyper™ platform which can also be adapted for MALDI tissue imaging applications.
- Easy to implement suitability tests for HTX sprayer and timsTOFfleX to show when system requires maintenance.
- QC arrays to track MALDI-MS performance, and negative and positive controls serve as lower and upper reference boundaries for patient

Imaging MS: Instrumentation