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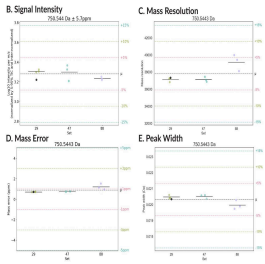
QC Metrics for KPMP Data Collection by VU TIS



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Katerina V Djambazova[1], Lukasz Migas[2], Jamie Allen[1], Madeline E. Colley[1], Martin Dufrense[1], Allison Esselman[1], Léonore Tideman[2], Ellie Pringy[1], Angela R.S. Kruse[1], Melissa Farrow[1], Raf Van De Plas[2], Jeff Spraggins[1]

[1]Vanderbilt University; [2]Delft University of Technology

VU Biomolecular Multimo… KPMP

Katerina V Djambazova
Vanderbilt University

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We use this protocol and it's working

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Abstract

This protocol describes the Go/No-Go criteria, QC/QA steps, and metrics used in the "Multimodal Molecular Imaging" pipeline by the Vanderbilt University Tissue Interrogation Site (VU TIS) as part of the Kidney Precision Medicine Project (KPMP).

Biopsy Screening & Go/No-Go Criteria

1 To accept KPMP samples for analysis, the following sample processing Go/No-Go criteria in **Table 1** must be met:

Table 1. Sample Processing Go/No-Go Criteria

	A	B
	Go/No-Go Criteria	**Reasoning**
	Biopsy must be LN2 core	Other storage conditions can induce analyte suppression effect in MALDI IMS analysis
	Biopsy size must be > 3mm	Smaller tissue sections are difficult to handle, section, and mount
	Time to preservation must be < 10 min	Prolonged exposure prior to preservation can cause metabolite/lipid degradation

Accepted biopsies will be cryo-sectioned to 10 µm thickness and visually assessed following the QC metrics described in **Table 2** before data collection.

Table 2. Tissue QC Metrics

	A	B
	Tissue QC	**Reasoning / Data Flags**
	Are there any glomeruli in the biopsy section?	Ensures proper coverage of biological features
	Are there visible cracks/folds on the tissue surface?	This can lower FTU segmentation performance, due to non-segmentable features
	Are there visible freezing artifacts on the tissue surface?	Freezing artifacts can induce analyte delocalization, jeopardizing MALDI IMS spatial integrity

Autofluorescence Microscopy & FTU Segmentation Performance

2 Autofluorescence microscopy QC is performed prior to collecting autofluorescence data from KPMP biopsies. A detailed protocol for Autofluorescence Microscopy QC data collection is included **here**.

The mean fluorescence intensity (above the noise level) is calculated for each channel (eGFP, DAPI, DsRed) and monitored over time. To pass QC, intensity should be within ±15% of the mean for each channel.

3 Autofluorescence-based FTU segmentation is performed on each KPMP biopsy and for one reference tissue, termed *BlockQC*.

Percent coverage is calculated for each tissue section. A coverage >60% meets QC standards. When lower segmentation coverage is observed, the data is flagged for re-evaluation.

Imaging Mass Spectrometry

4 **MALDI IMS Data Collection**

Mass calibration is performed prior to MALDI IMS data collection. The mass must be calibrated to <1 ppm error, corresponding to a 95% calibration score in the software.
Mass calibration and subsequent MALDI IMS data are also collected on an external reference tissue section (*BlockQC*).

5 **MALDI IMS Data Analysis**

Global Parameter Monitoring

Table 3 Summarizes the QC metrics for tracking the global performance of MALDI IMS. QC metrics for LC-MS/MS data analysis are also included in the table.

Table 3. QC Metrics for MALDI IMS Global Parameters

	A	B	C
	Parameter	**QC Threshold**	**Notes**
	Mean intensity variability*	± 15%	*BlockQC* section: Significant deviation from the mean intensity may suggest problems with the instrumentation. Instrument performance will be assessed.
	Number of detected peaks*	> 600 (positive mode) > 900 (negativemode)	Adaptive S/N thresholding is applied until the minimal acceptable peaks are detected.
	Number of annotated peaks	~ 270 (positive mode) ~ 450 (negative mode)	Expected range, but real numbers may vary, especially in KPMP biopsies with CKD or AKI.
	Common annotated peaks	> 150 (positive mode) > 220 (negative mode)	Expected range, but real numbers may vary, especially in KPMP biopsies with CKD or AKI.
	LC-MS/MS	> 300 (positive mode) > 300 (negative mode)	Expected range, but real numbers may vary, especially in KPMP biopsies with CKD or AKI.

**Denotes critical data flags in the workflow that require re-processing*

Individual Ion Monitoring

For a set of diagnostic ions (in each polarity), we will monitor five parameters Listed in **Table 4**.

Table 4. QC Metrics for MALDI IMS Individual Ion Parameters

	A	B	C
	Parameter	**QC Threshold**	**Notes**
	Peak Alignment	-	Visual interpretation and marked for pre-processing if not well aligned. Realignment may be performed.
	Peak Width	± 10%	Expected deviation, however, real values may vary for lower intensity species.
	Mass Resolving Power	± 10%	Significant deviations may be indicative of compromised instrument performance. Instrument performance will be assessed.
	Peak Intensity	± 15%	*BlockQC*: We allow for a wider peak intensity variability, as even within the same tissue block, analyte intensity is highly dependent on the sampled structures. KPMP biopsies: CKD and AKI may significantly impact the peak intensity of the monitored panel of ions; therefore no meaningful boundary can be estimated here.
	Mass Error*	± 5 ppm	*BlockQC*: If abnormally high ppm errors are detected for the set panel of individual ions, the data are flagged for pre-processing. Individual datasets can be recalibrated (internally) with different ions.

**Denotes critical data flags in the workflow that require re-processing*

Global parameters and individual ion monitoring is performed on both KPMP samples as well as for the external reference tissue.

QC Data Collection & Recording

6 The collected QC data will be compiled in Levy-Jennings plots to longitudinally track the performance of the assays. QC data for both the KPMP samples and the reference tissue are summarized and reported.

Protocol references

Include workflow references when available.