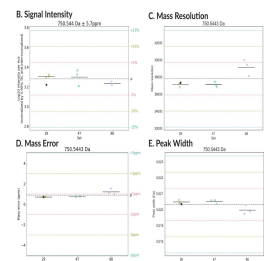


Feb 26, 2025

QC Metrics for KPMP Data Collection by VU TIS

DOI

<https://dx.doi.org/10.17504/protocols.io.14egn3wqyl5d/v1>



Katerina Djambazova¹, Lukasz Migas², Jamie Allen¹, Madeline E. Colley¹, Allison Esselman¹, Léonore ideman², Ellie Pringy¹, Angela R.S. Kruse¹, Melissa Farrow¹, Raf Van De Plas², Jeff Spraggins¹, Martin Dufrense¹

¹Vanderbilt University; ²Delft University of Technology

VU Biomolecular Multim...

KPMP



Katerina V Djambazova

Vanderbilt University

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.14egn3wqyl5d/v1>

Protocol Citation: Katerina Djambazova, Lukasz Migas, Jamie Allen, Madeline E. Colley, Allison Esselman, Léonore ideman, Ellie Pringy, Angela R.S. Kruse, Melissa Farrow, Raf Van De Plas, Jeff Spraggins, Martin Dufrense 2025. QC Metrics for KPMP Data Collection by VU TIS. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.14egn3wqyl5d/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: October 12, 2023

Last Modified: February 26, 2025

Protocol Integer ID: 89175

Keywords: qc metrics for kmp data collection, qc metric, part of the kidney precision medicine project, kidney precision medicine project, kmp data collection, pipeline by the vanderbilt university tissue interrogation site, vanderbilt university tissue interrogation site, multimodal molecular imaging, qc, kmp, vu tis this protocol, vu ti, qa step, go criteria, metric

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 μ m 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 $\pm 15\%$ 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*	$\pm 15\%$	<i>BlockQC</i> 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 (negative mode)	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	Expected range, but real numbers may vary, especially in KPMP biopsies with

A	B	C
	(negative mode)	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	$\pm 10\%$	Expected deviation, however, real values may vary for lower intensity species.
Mass Resolving Power	$\pm 10\%$	Significant deviations may be indicative of compromised instrument performance. Instrument performance will be assessed.
Peak Intensity	$\pm 15\%$	<i>BlockQC</i> : 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	<i>BlockQC</i> : 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

- 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.