The mass spectrometers are tuned as defined based on the particular platform in use, assay performance requirements, and specimen types tested.

NOTE: Instruments must be tuned at least as frequently as recommended by the manufacturer. Acceptable tolerance limits for tune parameters must be defined, and tuning records retained.

Tandem mass spectrometers require tuning at the time of maintenance that requires shutdown of the vacuum.

Evidence of Compliance:
- ✓ Records of tuning

*****NEW** 08/24/2023***

CBG.17150 Validation, Monitoring, and Annual Verification of MS Data Analysis Tools Phase II

The laboratory validates data analysis tools used for compound identification and quantification when first installed and after any modifications, as applicable, and verifies performance at least annually.

NOTE: Data analysis tools may be used for various processes, such as integration of targeted and untargeted peaks, evaluating acceptability of calibration and control performance, stability of baseline, calculation of ion mass ratios, discrimination of positive and negative results, and assessing risk of carryover. Data analysis tools (eg, software or code-based rules, algorithms, machine learning) used for automated data analysis must be verified using defined acceptability criteria. Version control of custom data analysis tools is required. Reassessment of lower limit of quantification (LLOQ) and other decision points may be used to ensure that a shift has not occurred due to instrument performance or another factor impacting assay performance.

Customized data analysis tools, and modifications to that software, should be appropriately documented and records should allow for tracking to identify persons that have added or modified that software. The purpose of the computer program, the way it functions, and its interaction with other programs must be clearly stated. The level of detail should be adequate to support troubleshooting, system modifications, or additional programming.

Evidence of Compliance:
- ✓ Records of validation and revalidation after modifications **AND**
- ✓ Records of monitoring for changes to software update tools and other change impacting performance

REFERENCES

1) Vincente FB, Lin DC, Haymond S. Automation of chromatographic peak review and order to result data transfer in a clinical mass spectrometry laboratory. *Clin Chim Acta.* 2019;498(11):84-9.

CBG.17300 Identification Criteria - Single Stage Mass Spectrometry Phase II

The identification criteria for single stage mass spectrometry (ie, GC/MS, LC/MS) are in compliance with recommendations.

NOTE: One acceptable criterion for compound identification by GC/MS using ion ratios is that the unknown result must have ion ratios within a predefined tolerance limit. This limit should be supported by either literature references or through experimental means. Such ion ratio tolerance limits may differ based on the technique applied (eg, GC/MS versus LC/MS) as well as the analyte(s) being determined (eg, compounds with mainly ions of low abundance); thus, a defined limit to cover all methods and analytes cannot be given.

Identification using ion ratios typically requires the use of at least two ion ratios. However, one ion ratio of two characteristic ions may be acceptable if there are only a few characteristic ratios AND if there are other identifying characteristics, eg, retention time. The internal standard's identification should be monitored with at least one ion ratio. An acceptable criterion for compound identification using total spectra is that the unknown result must have a "spectral

match" quality or fit that is within the defined limits that the laboratory has set and validated. Ion ratios determined from total spectra analysis are an acceptable identification method, and should fulfill the same criteria as given above for ion ratio identification.

Laboratories using mass spectrometric methods for quantitative purposes based on total ion current measurements (without ion ratios) should have ancillary information and assay characteristics that validate this process, eg, known compound of interest, retention times, potential interferences by endogenous compounds or other drugs/metabolites, etc.

Evidence of Compliance:
✓ QC and test records

REFERENCES
1) Clinical and Laboratory Standards Institute (CLSI). *Liquid Chromatography-Mass Spectrometry Methods.* 2nd ed. CLSI document C62. Clinical and Laboratory Standards Institute, Wayne, PA; 2022.

CBG.17400 Identification Criteria - Tandem Mass Spectrometry **Phase II**

The identification criteria for tandem mass spectrometry (MS/MS) are validated and recorded.

NOTE: In tandem mass spectrometry using multiple reaction monitoring (MRM) there is at least one transition monitored for the internal standard and another for the analyte.

Evidence of Compliance:
✓ QC and test records

***REVISED** 08/24/2023*
CBG.17500 Matrix Effect Assessment of Mass Spectrometry Assays - Validation **Phase II**

There is a record of assessment of matrix effects in development and validation of mass spectrometry assays.

NOTE: Matrix effects can affect analyte ionization and performance in both directions: suppression or, less frequently seen, enhancement. Evaluation of matrix effects must be performed during assay development and validation.

Examples of evaluation protocols may include:
1. *Post Column Infusion - Constant infusion of analyte followed by injection of blank matrix specimen extracts to measure ionization response*
2. *Mobile Phase/Post Extractions Spiking - Compare response of analyte spiked into mobile phase to that of analyte spiked into blank matrix specimen extracts*
3. *Internal standard monitoring - Evaluate trends in internal standard abundance and signal to noise ratios during an analytical run that includes blank and spiked matrix specimen extracts.*

The minimum number of different matrix sources may vary based on the matrix, analytical targets, and assay design. Associated data should be used to evaluate the impact of matrix effect on results and define appropriate acceptance criteria for each reportable analyte during routine testing of patient samples.

REFERENCES
1) Clinical and Laboratory Standards Institute. *Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance; Approved Guideline.* CLSI Document C50-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2007.
2) Clinical and Laboratory Standards Institute (CLSI). *Liquid Chromatography-Mass Spectrometry Methods.* 2nd ed. CLSI document C62. Clinical and Laboratory Standards Institute, Wayne, PA; 2022.
3) Annesley TM. Ion Suppression in Mass Spectrometry. *Clin. Chem.* 49, pp. 1041-1044 (2003).

***REVISED** 08/24/2023*
CBG.17600 Matrix Effect Assessment of Mass Spectrometry Assays - Routine Monitoring **Phase II**